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WRITER'S DIRECT NUMBER:

August 26, 1997

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**Box Patent Application**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Re: U.S. Non-Provisional Patent Application  
Appl. No. To be assigned; Filed: August 26, 1997  
For: **Soluble Interleukin-1 Receptor Accessory Molecule**  
Inventors: Daniel P. BEDNARIK, Henrik S. OLSEN, and Craig A. ROSEN  
Our Ref: 1488.0450001/EKS/KMT

Sir:

The following documents are forwarded herewith for appropriate action by the U.S. Patent and Trademark Office:

1. U.S. Utility Patent Application entitled:  
**Soluble Interleukin-1 Receptor Accessory Molecule**  
and naming as inventors:  
  
Daniel P. BEDNARIK  
Henrik S. OLSEN  
Craig A. ROSEN  
  
the application consisting of:

269230 "DT 227680"

Assistant Commissioner for Patents

August 26, 1997

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a. A specification containing:

(i) 77 pages of description prior to the claims, including a sequence listing on pages 60 to 77;

(ii) 6 pages of claims (19 claims);

(iii) a one (1) page abstract;

b. 4 sheets of drawings: (Figures 1, 2A, 2B and 3);

c. A computer readable copy of the sequence listing; and

2. Two (2) return postcards.

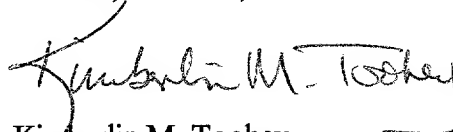
It is respectfully requested that, of the two attached postcards, one be stamped with the filing date of these documents and returned to our courier, and the other, prepaid postcard, be stamped with the filing date and unofficial application number and returned as soon as possible.

This patent application is being submitted under 37 C.F.R. § 1.53(b)(1) without Declaration and without filing fee.

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the sequence listing and the computer readable copy of the sequence listing submitted herewith in the above application are the same.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



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08947710-082697

## **Soluble Interleukin-1 Receptor Accessory Molecule**

This application claims the benefit of the filing date of provisional application 60/024,581 filed on August 26, 1996, which is herein incorporated by reference.

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### ***Field of the Invention***

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The present invention relates to a novel soluble Interleukin-1 receptor accessory molecule (IL-1R AcM). IL-1R AcM is a member of the Ig superfamily by analysis of its putative extracellular domain and bears limited homology throughout the protein to both Type I and Type II IL-1 receptors. More specifically, isolated nucleic acid molecules are provided encoding a human microvascular endothelial-derived soluble IL-1R AcM. The IL-1R AcM polypeptides are also provided. The present invention further relates to screening methods for putative agonists and antagonists of IL-1 signal transduction.

### ***Background of the Invention***

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Interleukin 1 (IL-1) is a polypeptide cytokine with multiple diverse effects on immunological and inflammatory processes. While many of the roles of IL-1 in inflammation and the immune response have been well characterized, the molecular basis of these responses remains unclear (reviewed by Dinarello, *Blood*, 77: 1627-1652). IL-1 is produced by a diversity of cell types and elicits a wide variety of physiological effects in hematopoietic and nonhematopoietic cells. Thus, IL-1 has biological effects on hematopoietic cells, the digestive tract, bone, cartilage and connective tissue, vascular cells, the skin, the endocrine system, the gonads, and on neural tissue. In addition, IL-1 is produced by malignant cells. (Pimentel, *Handbook of Growth Factors: Volume III Hematopoietic Growth Factors and Cytokines*, pp. 35-53, CRC Press, Boca Raton, FL 1994).

The IL-1 family of proteins comprises three members: IL-1 $\alpha$  and IL-1 $\beta$  (capable of inducing IL-1 biological responses) and IL-1ra (a pure receptor antagonist). These ligands bind to two distinct and separate receptors: the Type I and Type II IL-1 receptors (IL-1Rs). The 80-kD Type I IL-1R is found mainly on  
5 T cells and fibroblasts (Sims, J.E., *et al.*, *Science* 241:585-589 (1988); Chizzonite, R., *et al.*, *Proc. Natl. Acad. Sci. USA* 86:8029-8033 (1989); Sims, J.E., *et al.*, *Proc. Natl. Acad. Sci. USA* 86:8946-8950 (1989)). The 68-kD Type II IL-1R is found predominantly on B cells and neutrophils (Chizzonite, R., *et al.*, *Proc. Natl. Acad. Sci. USA* 86:8029-8033 (1989); Sims, J.E., *et al.*, *Proc. Natl.*  
10 *Acad. Sci. USA* 86:8946-8950 (1989); McMahan, C.J., *et al.*, *EMBO J.* 10:2821-2832 (1991)). Both receptor types contain a large cytoplasmic region, a single transmembrane domain, and three extracellular Ig-like domains, a structural organization that classifies them as members of the Ig superfamily. The Type I IL-1R has a cytoplasmic tail of approximately 200 amino acids, while the Type II  
15 IL-1R cytoplasmic tail is only 29 amino acids. The agonists IL-1 $\alpha$  and IL-1 $\beta$  bind to the extracellular domains of both receptors, although with different affinities (reviewed in Dower *et al.*, *Cellular and Molecular Mechanisms of Inflammation*, pp. 137-172, Academic Press, Orlando FL.).

The relative importance of the Type I and Type II IL-1Rs in IL-1 signaling  
20 has been recently clarified. A critical role for the Type I IL-1R in IL-1-induced activation of NF- $\kappa$ B, IL-6, and IL-8 secretion, and cell adhesion molecule expression has been demonstrated by several groups (Stylianou, E., *et al.*, *J. Biol. Chem.* 267:15836-15841 (1992); Colotta, F., *et al.*, *Science* 261:472-475 (1993); Sims, J.E., *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6155-6159 (1993)). In contrast,  
25 the Type II IL-1R appears to be dispensable for IL-1 signaling and may act as a decoy receptor (Stylianou, E., *et al.*, *J. Biol. Chem.* 267:15836-15841 (1992); Colotta, F., *et al.*, *Science* 261:472-475 (1993); Sims, J.E., *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6155-6159 (1993)). While it appears clear that the Type I IL-1R is necessary for IL-1 signal transduction, it is uncertain if it is the only cell-  
30 surface molecule involved in IL-1 signaling.

It has been assumed that the functional Type I IL-1R is a single chain receptor (Curtis, B.M., *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:3045-3049 (1989)). However, affinity cross-linking of IL-1 to cells expressing natural IL-1 receptor has yielded complex patterns of cross-linked proteins (Dower, *et al.*, *Cellular and Molecular Mechanisms of Inflammation*, pp. 137-172, Academic Press, Orlando FL (1990); Dinarello, *et al.*, *Immunol. Today*, 10: 49-51 (1989)). These cross-linking studies detect molecular mass complexes consistent with both the Type I and Type II IL-1Rs cross-linked to IL-1. In addition, in some studies, higher molecular mass complexes (>200 kD) are apparent (Kupper, T.S., *et al.*, *J. Clin. Invest.* 82:1787-1792 (1988); Dinarello, C.A., *et al.*, *Immunol. Today* 10:49-51 (1989); Solari, R., *Cytokine* 2:21-28 (1990); Mancilla, J., *et al.*, *Lymph. Cytokine Res.* 11:197-205 (1992)). Some reports have interpreted these higher molecular mass complexes to be dimmers of receptor-ligand complexes. Others have concluded that these high molecular mass complexes maybe indicative of a multi-subunit IL-1 receptor complex.

Only two IL-1R accessory proteins are have been identified. Studies initiated to identify components of a potential IL-1 receptor complex suggest that there is a cell-surface protein in close association with the IL-1R that may play a role in IL-1 receptor binding and signaling. A murine IL-1 receptor accessory protein (mulL-1R AcP) has been cloned and expressed (Greenfeder *et al.* *J. Biol.Chem.* 270: 13757-13765 (1995)) . This protein was present in brain, lung, spleen, and thymus tissues. A search of the GenBank data base with the mulL-1R AcP cDNA sequence revealed significant homology (82%) to a cDNA isolated from human infant brain (accession no. T08277) (Adams, M.D., *et al.*, *Nature Genet.* 4:373-380 (1993)). No other significant homologies were found in GenBank. The reported sequence for this partial cDNA is 396 bp long and represents one of 1600 cDNAs that were sequenced from a library made to contain only expressed sequence tags. The regions to overlap with the mulL-1R AcP sequence is nucleotides 893-1286 of the mulL-R AcP, which include the transmembrane domain. Although Adams *et al.* (Adams, M.D., *et al.*, *Nature Genet.* 4:373-380 (1993)) assigned no function to this partial cDNA, it is likely

that it encodes a portion of a human homologue of muIL-1R AcP. Using the muIL-1R AcP cDNA has >95% homology to the partial sequence of Adams *et al.* and ~90% homology to the muIL-1R AcP cDNA. This partial cDNA was isolated as an expressed gene in infant brain. This was consistent with Northern analysis results of Greenfeder *et al.* demonstrating that muIL-1R AcP mRNA is constitutively expressed at high levels in mouse brain.

The discovery of IL-1R accessory molecule has a number of implications for IL-1 receptor biology. First, while muIL-1R AcM may not bind IL-1 directly, the accessory molecule forms a complex with the muType I IL-1R allowing IL-1 $\beta$  to bind with higher affinity than the muType I IL-1R alone (Greenfeder, *et al. J. Biol. Chem.*, 270: 13757-13765 (1995)). Thus, the presence or absence of the accessory molecule in different cell lines determined whether the low or the higher affinity site was detected, suggesting that the low affinity site corresponds to the muType I IL-1R alone, while the higher affinity site represents a complex of the muType I IL-1R with the muIL-1R AcM. (Greenfeder, *et al. J. Biol. Chem.*, 270: 13757-13765 (1995)). In this respect, the IL-1R AcM would be analogous to affinity conversion and signal transduction subunits such as gp 130 in the IL-6 system (Hibi, M., *et al., Cell* 63:1149-1157 (1990)), the common  $\beta$  chain of the IL-3, granulocyte/macrophage colony-stimulating factor, and IL-5 receptors (Kitamura, T., *et al., Cell* 66:1165-1174 (1991)), and the  $\gamma_c$  subunit first identified as part of the IL-2 receptor (reviewed in Minami, *et al., Annu. Rev. Immunol.* 11: 245-267 (1993)).

Second, the possible existence of a multi-subunit IL-1 receptor complex contradicts a previous hypothesis that the Type I IL-1R is the entire functional receptor for IL-1 signaling (Dower, S.K. & Sims, J.E., *Cellular and Molecular Mechanisms of Inflammation*, Academic Press, Orlando, FL (1990), pp. 137-172; Curtis, B.M., *et al., Proc. Natl. Acad. Sci. USA*, 86:3045-3049 (1989)). This hypothesis was based on the observation that CHO cells expressing recombinant murine Type I IL-1R were more sensitive than control CHO cells to low concentrations of IL-1, and that the increase in sensitivity was proportional to the number of murine Type I IL-1Rs (Curtis, B.M., *et al., Proc. Natl. Acad. Sci. USA*,

86:3045-3049 (1989)). An alternative explanation for these results is that the endogenous hamster IL-1R, was able to form a functional receptor complex with the mu Type I IL-1R, thus enhancing IL-1 signaling in the transfected cells (Greenfeder, *et al. J. Biol. Chem.*, 270: 13757-13765 (1995)).

5 Third, the discovery of the accessory protein provides an intriguing explanation for the antagonist activity of IL-1ra despite its high affinity binding to the Type I IL-1R. The inability of IL-1ra to interact with the muIL-1R AcP, the putative signal transducing subunit of the IL-1R complex, would result in the absence of a biological response.

### 10 *Summary of the Invention*

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding the soluble IL-1R AcM polypeptide having the amino acid sequence is shown in Figure 1 [SEQ ID NO:2] or the amino acid sequence encoded by the cDNA clone deposited in a bacterial host as ATCC Deposit  
15 Number 97666 on July 25, 1996. The nucleotide sequence determined by sequencing the deposited IL-1R AcM clone, which is shown in Figure 1 [SEQ ID NO:1], contains an open reading frame encoding a polypeptide of 356 amino acid residues, including an initiation codon at positions 303-305, with a leader sequence of about 17 amino acid residues, and a predicted molecular weight of  
20 about 42 kDa. The amino acid sequence of the mature IL-1R AcM protein is amino acid residues 18-356 shown in Figure 1 or 1-339 shown in SEQ ID NO:2.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors  
25 and host cells and for using them for production of soluble IL-1R AcM polypeptides or peptides by recombinant techniques.

The invention further provides an isolated soluble IL-1R AcM polypeptide having amino acid sequence encoded by a polynucleotide described herein.

The soluble IL-1R AcM may not bind IL-1 directly, however, the accessory molecule forms a complex with the Type I IL-1R that binds IL-1 $\beta$  with higher affinity than the Type I IL-1R alone. Thus, the presence or absence of the accessory molecule in different cell lines determines whether the low or the higher affinity site is detected suggesting that the low affinity site corresponds to the Type I IL-1R alone, while the higher affinity site represents a complex of the Type I IL-1R with the IL-1R AcM. The present invention further provides a screening method for identifying IL-1 receptor agonists and antagonists, which involves: (a) providing a polypeptide comprising a type I IL-1 receptor and a polypeptide comprising IL-1R AcM or IL-1R AcM fragment, wherein IL-1R and IL-1R AcM or IL-1R and the IL-1R AcM fragment form a complex; (b) providing a candidate compound; (c) providing a polypeptide comprising IL-1 or a functional IL-1 fragment; and (d) determining the binding affinity of said complex for IL-1 whereby an increased binding affinity of said complex for IL-1 in the presence of said compound is indicative that said compound is an agonist for IL-1 signal transduction and a decreased binding affinity of said complex for IL-1 in the presence of said compound is indicative that said compound is an antagonist of IL-1 signal transduction.

### ***Brief Description of the Figures***

Figure 1 shows the nucleotide [SEQ ID NO:1] and deduced amino acid [SEQ ID NO:2] sequences of soluble IL-1R AcM. The protein has a leader sequence of about 17 amino acid residues (underlined) and a deduced molecular weight of about 42 kDa. The predicted amino acid sequence of the mature soluble IL-1R AcM protein is also shown in Figure 1 [SEQ ID NO:2].

Figures 2A and B shows the regions of similarity between the amino acid sequences of the soluble IL-1R AcM protein (HMEEJ22) and mouse interleukin 1 receptor accessory protein [SEQ ID NO:3] and between the amino acid sequences of the soluble IL-1R AcM protein (HMEEJ22) and the partial cDNA



isolated from human infant brain (Adams, M.D., et al., Nature Genet. 4:373-380 (1993)) [SEQ ID NO:4].

Figure 3 shows an analysis of the IL-1R AcM amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph (boxed graph), the amino acid sequence of the IL-1R AcM protein is shown with the amino acids that border each peak from the antigenic index plot displayed as underlined characters.

### *Detailed Description*

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding soluble IL-1R AcM polypeptide, having the amino acid sequence shown in Figure 1 [SEQ ID NO:2], which was determined by sequencing a cloned cDNA. The soluble IL-1R AcM protein of the present invention shares sequence homology with mouse interleukin 1 receptor accessory protein (Figure 2) [SEQ ID NO:3]. The nucleotide sequence shown in Figure 1 [SEQ ID NO:1] was obtained by sequencing the HMEEJ22 clone, which was deposited on July 25, 1996 at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, and given accession number 97666. The deposited clone is inserted in the pBluescript SK(-) plasmid (Stratagene, La Jolla, CA).

Accordingly, in one embodiment of the present invention, isolated nucleic acid molecules are provided which encode the soluble IL-1R AcM protein. The IL-1R AcM is a novel member of the Ig superfamily.

### *Nucleic Acid Molecules*

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA

sequencer (such as the Model 373 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Using the information provided herein, such as the nucleotide sequence in Figure 1, a nucleic acid molecule of the present invention encoding a soluble IL-1R AcM polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in Figure 1 [SEQ ID NO:1] was discovered in a cDNA library derived from human microvascular endothelial cells. The determined nucleotide sequence of the soluble IL-1R AcM cDNA of Figure 1 [SEQ ID NO:1] contains an open reading frame encoding a protein of 356 amino acid residues, with an initiation codon at positions 303-306 of the nucleotide sequence in Figure 1 [SEQ ID NO:1], a predicted leader sequence of about 17 amino acid residues, and a deduced molecular weight of about 42 kDa. The amino acid sequence of the predicted mature soluble IL-1R AcM is amino acid residue 18 to residue 356 shown in Figure 1 or amino acids 1-339 shown in SEQ ID NO:2. The soluble IL-1R AcM protein shown in Figure 1 [SEQ ID NO:2] is about 94% similar and 85% identical to mouse interleukin

1 accessory protein (Figure 2A). In addition, the nucleotides 1060 to 1353 of  
soluble IL-1R AcM protein shown in Figure 1 [SEQ ID NO:2] is about 99%  
similar and 98% identical to the first 294 nucleotides partial cDNA isolated from  
human infant brain by Adams, M.D., *et al.*, *Nature Genet.* 4:373-380 (1993) [SEQ  
ID NO:4](Figure 2B). The partial cDNA isolated by Adams was 396 nucleotides  
in length.

The present invention also provides the mature form(s) of the soluble IL-  
1R AcM protein of the present invention. According to the signal hypothesis,  
proteins secreted by mammalian cells have a signal or secretory leader sequence  
which is cleaved from the mature protein once export of the growing protein  
chain across the rough endoplasmic reticulum has been initiated. Most  
mammalian cells and even insect cells cleave secreted proteins with the same  
specificity. However, in some cases, cleavage of a secreted protein is not entirely  
uniform, which results in two or more mature species on the protein. Further, it  
has long been known that the cleavage specificity of a secreted protein is  
ultimately determined by the primary structure of the complete protein, that is, it  
is inherent in the amino acid sequence of the polypeptide. Therefore, the present  
invention provides a nucleotide sequence encoding the mature soluble IL-1R  
AcM polypeptides having the amino acid sequence encoded by the cDNA clone  
contained in the host identified as ATCC Deposit No. 97666 and as shown in  
SEQ ID NO:2. By the mature soluble IL-1R AcM protein having the amino acid  
sequence encoded by the cDNA clone contained in the host identified as ATCC  
Deposit 97666 is meant the mature form(s) of the soluble IL-1R AcM protein  
produced by expression in a mammalian cell (e.g., COS cells, as described below)  
of the complete open reading frame encoded by the human DNA sequence of the  
clone contained in the vector in the deposited host. As indicated below, the  
mature soluble IL-1R AcM having the amino acid sequence encoded by the  
cDNA clone contained in ATCC Deposit No. 97666 may or may not differ from  
the predicted "mature" soluble IL-1R AcM protein shown in SEQ ID NO:2  
(amino acids from about 1 to about 339) depending on the accuracy of the  
predicted cleavage site based on computer analysis.

Methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the methods of McGeoch (*Virus Res.* 3:271-286 (1985)) and von Heinje (*Nucleic Acids Res.* 14:4683-4690 (1986)) can be used. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. von Heinje, *supra*. However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the predicted amino acid sequence of the complete soluble IL-1R AcM polypeptides of the present invention were analyzed by a computer program ("PSORT") (K. Nakai and M. Kanehisa, *Genomics* 14:897-911 (1992)), which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis by the PSORT program predicted the cleavage site between amino acids -1 and 1 in SEQ ID NO:2. Thereafter, the complete amino acid sequences were further analyzed by visual inspection, applying a simple form of the (-1,-3) rule of von Heinje. von Heinje, *supra*. Thus, the leader sequence for the soluble IL-1R AcM protein is predicted to consist of amino acid residues from about - 17 to about - 1 in SEQ ID NO:2, while the mature soluble IL-1R AcM protein is predicted to consist of residues from about 1 to about 339.

As one of ordinary skill would appreciate, due to the possibilities of sequencing errors discussed above, as well as the variability of cleavage sites for leaders in different known proteins, the actual soluble IL-1R AcM polypeptide encoded by the deposited cDNA comprises about 356 amino acids, but may be anywhere in the range of 345-370 amino acids; and the actual leader sequence of this protein is about 17 amino acids, but may be anywhere in the range of about 10 to about 20 amino acids.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The

DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) with an initiation codon at positions 303-306 of the nucleotide sequence shown in Figure 1 [SEQ ID NO:1]; DNA molecules comprising the coding sequence for the mature soluble IL-1R AcM protein shown in Figure 1 (last 339 amino acids) [SEQ ID NO:2]; and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the soluble IL-1R AcM protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate the degenerate variants described above.

In addition, the invention provides nucleic acid molecules having nucleotide sequences related to extensive portions of SEQ ID NO:1 which have been determined from the following related cDNA clones: HE8MI45R (SEQ ID NO:5), HWEBD79F (SEQ ID NO:6), HSJBY21R (SEQ ID NO:7), HCE4Z93R (SEQ ID NO:8), HTEBZ03RA (SEQ ID NO:9).

Sequences of public ESTs that relate to a portion of SEQ ID NO:1 have the following GenBank Accession Numbers: T70598 (SEQ ID NO:10), W85847 (SEQ ID NO:11), T83863 (SEQ ID NO:12), T08277 (SEQ ID NO:13), T70863 (SEQ ID NO:14), H80590 (SEQ ID NO:15), H80533 (SEQ ID NO:16), R35902

(SEQ ID NO:17), T91161 (SEQ ID NO:18), D79417 (SEQ ID NO:19), R35903 (SEQ ID NO:20), R78680 (SEQ ID NO:21) and W85846 (SEQ ID NO:22).

In another aspect, the invention provides isolated nucleic acid molecules encoding the soluble IL-1R AcM polypeptide having an amino acid sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 97666 on July 25, 1996. In a further embodiment, nucleic acid molecules are provided encoding the mature soluble IL-1R AcM polypeptide or the full-length soluble IL-1R AcM polypeptide lacking the N-terminal methionine. The invention also provides an isolated nucleic acid molecule will encode the mature polypeptide encoded by the above-described deposited cDNA clone. The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in Figure 1 [SEQ ID NO:1] or the nucleotide sequence of the soluble IL-1R AcM cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the soluble IL-1R AcM gene in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequence shown in SEQ ID NO:1 is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975, 1000, 1500 or 2100 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in SEQ ID NO:1. By a fragment at least 20 nt in length, for example, is intended fragments which

include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in SEQ ID NO:1.

In another aspect, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clone contained in ATCC Deposit 97666. By "stringent hybridization conditions" is intended overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150 mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30-70 nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in SEQ ID NO:1). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the soluble IL-1R AcM cDNA shown in SEQ ID NO:1), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

Since a soluble IL-1R AcM cDNA clone has been deposited and its determined nucleotide sequence is provided in Figure 1 [SEQ ID NO:1], generating polynucleotides which hybridize to a portion of the soluble IL-1R

AcM cDNA molecule would be routine to the skilled artisan. For example, restriction endonuclease cleavage or shearing by sonication of the soluble IL-1R AcM cDNA clone could easily be used to generate DNA portions of various sizes which are polynucleotides that hybridize to a portion of the soluble IL-1R AcM cDNA molecule. Alternatively, the hybridizing polynucleotides of the present invention could be generated synthetically according to known techniques. Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the soluble IL-1R AcM cDNA shown in Figure 1 [SEQ ID NO:1]), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding epitope-bearing portions of the soluble IL-1R AcM protein. In particular, isolated nucleic acid molecules are provided encoding polypeptides comprising the following amino acid residues in Figure 1 (SEQ ID NO:2), which the present inventors have determined are antigenic regions of the soluble IL-1R AcM protein: In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 6 to about 15 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 57 to about 66 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 70 to about 79 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 106 to about 112 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 115 to about 124 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 129 to about 135 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 158 to about 172 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 180 to about 187 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 207 to about 215 in SEQ ID NO:2; a polypeptide comprising amino acid residues from



about 231 to about 244 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 247 to about 255 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 268 to about 276 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 285 to about 295 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 303 to about 310 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 319 to about 330 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from about 333 to about 339 in SEQ ID NO:2. Methods for generating such epitope-bearing portions of soluble IL-1R AcM are described in detail below.

As indicated, nucleic acid molecules of the present invention which encode a soluble IL-1R AcM polypeptide may include, but are not limited to those encoding the amino acid sequence of the mature polypeptide, by itself; the coding sequence for the mature polypeptide and additional sequences, such as those encoding the about 17 amino acid leader or secretory sequence, such as a pre-, or pro- or prepro- protein sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which

corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson *et al.*, *Cell* 37: 767 (1984).. As discussed below, other such fusion proteins include the soluble IL-1R AcM fused to Fc at the N- or C-terminus.

5           The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the soluble IL-1R AcM protein. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*,  
10       Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

          Such variants include those produced by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding regions, non-coding  
15       regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the soluble IL-1R AcM protein or portions thereof. Also especially preferred in this regard are conservative  
20       substitutions. Most highly preferred are nucleic acid molecules encoding the mature protein having the amino acid sequence shown in Figure 1 [SEQ ID NO:2] or the mature soluble IL-1R AcM amino acid sequence encoded by the deposited cDNA clone.

          Further embodiments of the invention include isolated nucleic acid  
25       molecules comprising a polynucleotide having a nucleotide sequence at least 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2, but lacking the N-terminal  
30       methionine; (c) a nucleotide sequence encoding the polypeptide having the amino acid sequence at positions from about 1 to about 339 in SEQ ID NO:2; (d) a

nucleotide sequence encoding the polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97666; (e) a nucleotide sequence encoding the mature soluble IL-1R AcM polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit  
5 No. 97666; or (f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d) or (e).

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a soluble IL-1R AcM polypeptide is intended that the nucleotide sequence of the polynucleotide  
10 is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the soluble IL-1R AcM polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the  
15 reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either  
20 individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in Figure 1 or to the nucleotides sequence of the deposited  
25 cDNA clone can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2: 482-489 (1981), to find the  
30 best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is,

for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 [SEQ ID NO:1] or to the nucleic acid sequence of the deposited cDNA, irrespective of whether they encode a polypeptide having soluble IL-1R AcM activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having soluble IL-1R AcM activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having soluble IL-1R AcM activity include, *inter alia*, (1) isolating the soluble IL-1R AcM gene or allelic variants thereof in a cDNA library; (2) *in situ* hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the soluble IL-1R AcM gene, as described in Verma *et al.*, *Human Chromosomes: A Manual of Basic Techniques*, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting soluble IL-1R AcM mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 97%, 98% or 99% identical to the nucleic acid sequence shown in Figure 1 [SEQ ID NO:1] or to the nucleic acid sequence of the deposited cDNA which do, in fact, encode a polypeptide having soluble IL-1R AcM protein activity. By "a polypeptide having soluble IL-1R AcM activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to an activity of the soluble IL-1R AcM protein of the invention (either the full-length protein or, preferably, the mature protein), as measured in a particular biological assay. Assays of IL-1R AcM protein activity are well-known to those in the art.

These assays can be used to measure IL-1R AcM protein activity of partially purified or purified native or recombinant protein. For example, an equilibrium and competitive binding studies using CHO stable cell lines (Greenfeder *et al.*, *J. Biol. Chem.* 270: 13757-13765 (1995)) can be performed to detect IL-1R AcM activity.

For this assay, a CHO-IR/AcM cell line is established by simultaneous cotransfection of two expression vectors such that both IL-1R and IL-1R AcM or a candidate IL-1R AcM are expressed at about a 1:10 ratio of molecules/cell. In addition, control cell lines which express only IL-1R or IL-1R AcM are also established. To establish the stable cell lines, CHO-dhfr<sup>r</sup> cells are maintained in DMEM with 10% fetal bovine serum, 25mM HEPES, pH 7.0, 0.1 mM glutamine, 1X HT supplement (0.1 mM hypoxanthine, 0.016 mM thymidine) (Boehringer Mannheim), 50 µg/ml gentamicin, 1X penicillin/streptomycin/fungizone (JRH Biosciences). Cells are transfected with pSV2-dhfr (Subramani, *et al. Mol. Cell. Biol.* 1: 845-864 (1981)) either alone or in combination with expression vectors containing IL-1R, IL-1R AcM protein and a candidate IL-1R AcM protein by the CaPO<sub>4</sub> method following the manufacturer's directions (Stratagene). After three days, cells are transferred to medium lacking HT and allowed to grow an additional two weeks. Transfectants are then subjected to gene amplification by growth in increasing doses of methotrexate (0.1-1.0 µM). Clones are isolated by limiting dilution and screened by equilibrium binding with <sup>125</sup>I-labeled IL-1, anti-IL-1R antibody or anti-IL-1R AcM antibody.

To characterize the number and affinities of IL-1β binding sites, each of the above generated cell lines are analyzed by equilibrium binding with <sup>125</sup>I-labeled IL-1β. Equilibrium binding of <sup>125</sup>I-labeled IL-1 to the cells can be performed as described by Mizel, *et al. J. Immunol.* 138: 2906-2912 (1987). <sup>125</sup>I labeling of IL-1β can be performed by methods well known to those skilled in the art, for example, as described by Chizzonite, *et al. J. Immunol.* 147:1548-1556 (1991).

The activity of the accessory protein, IL-1R AcM, can be examined in the binding of IL-1β to a CHO-IR/AcM cell line obtained above. It is desirable for

this cell line to express an excess amount of IL-1R AcM protein relative to IL-1R. Cell lines bearing only IL-1R AcM protein do not bind IL-1 $\beta$  and cell lines bearing only IL-R will bind IL-1 $\beta$  with low affinity (i.e., approximately  $K_D$  1.0-3.3nM). A CHO-IR/AcM cell line, bearing both the IL-1R and IL-1R AcM results in IL-1R having a higher affinity binding site (i.e., approximately  $K_D$  0.02-0.8 nM). Thus, one can monitor the presence of IL-1R AcM activity by testing whether the putative accessory protein interacts with the IL-1R so as to generate a high affinity IL-1 $\beta$  binding site. The IL-1R AcM protein of the present invention can serve as a reference for the assay for IL-1R AcM activity associated with the high affinity IL-1R binding state.

In addition to the above described assay, one can evaluate whether a candidate polypeptide has IL-1R AcM activity by performing simple binding kinetics can be measured to determine receptor affinity for the ligand (i.e., IL-1 $\beta$ ). Binding kinetic analysis experiments are well known to those skilled in the art (Chizzonite *et al.*, *Proc Natl. Acad. Sci.* 86:8029-8033 (1989); Mizel, *et al.*, *J. Immunol.* 138:2906-2912 (1987)). One can monitor the presence of IL-1R AcM activity by testing whether the putative accessory protein interacts with the IL-1R so as to result in high affinity binding of IL-1 $\beta$  to the receptor. The IL-1R AcM protein of the present invention can serve as a reference for the assay for IL-1R AcM activity associated with the high affinity IL-1R binding state.

Thus, "a polypeptide having soluble IL-1R AcM protein activity" includes polypeptides that exhibit IL-1R AcM activity, in the above-described assay. Although the degree of activity need not be identical to that of the IL-1R AcM protein, preferably, "a polypeptide having IL-1R AcM protein activity" will exhibit substantially similar activity as compared to the IL-1R AcM protein (i.e., the candidate polypeptide will exhibit greater activity or not more than about tenfold less and, preferably, not more than about ten-fold less activity relative to the reference IL-1R AcM protein).

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99%

identical to the nucleic acid sequence of the deposited cDNA or the nucleic acid sequence shown in Figure 1 [SEQ ID NO:1] will encode a polypeptide "having soluble IL-1R AcM protein activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having soluble IL-1R AcM protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990), wherein the authors indicate that are surprisingly tolerant of amino acid substitutions.

### ***Vectors and Host Cells***

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of soluble IL-1R AcM polypeptides or fragments thereof by recombinant techniques.

Recombinant constructs may be introduced into host cells using well known techniques such infection, transduction, transfection, transvection, electroporation and transformation. The vector may be, for example, a phage, plasmid, viral or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a

precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

Preferred are vectors comprising cis-acting control regions to the polynucleotide of interest. Appropriate trans-acting factors may be supplied by the host, supplied by a complementing vector or supplied by the vector itself upon introduction into the host.

In certain preferred embodiments in this regard, the vectors provide for specific expression, which may be inducible and/or cell type-specific. Particularly preferred among such vectors are those inducible by environmental factors that are easy to manipulate, such as temperature and nutrient additives.

Expression vectors useful in the present invention include chromosomal-, episomal- and virus-derived vectors, e.g., vectors derived from bacterial plasmids, bacteriophage, yeast episomes, yeast chromosomal elements, viruses such as baculoviruses, papova viruses, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as cosmids and phagemids.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac*, *trp* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of



appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture  
5 mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pA2, pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia.  
10 Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Among known bacterial promoters suitable for use in the present invention include the *E. coli lacI* and *lacZ* promoters, the T3 and T7 promoters, the *gpt* promoter, the lambda PR and PL promoters and the *trp* promoter. Suitable eukaryotic promoters include the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus (RSV),  
15 and metallothionein promoters, such as the mouse metallothionein-I promoter.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis  
20 *et al.*, *Basic Methods In Molecular Biology* (1986).

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act to increase transcriptional activity of a promoter  
25 in a given host cell-type. Examples of enhancers include the SV40 enhancer, which is located on the late side of the replication origin at bp 100 to 270, the  
30

cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. The signals may be endogenous to the polypeptide or they may be heterologous signals.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize receptors. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, hIL5-receptor has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et

al., *Journal of Molecular Recognition*, Vol. 8 52-58 (1995) and K. Johanson et al., *The Journal of Biological Chemistry*, Vol. 270, No. 16, pp 9459-9471 (1995).

The soluble IL-1R AcM protein can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

#### ***IL-1R AcM Polypeptides and Fragments***

The invention further provides an isolated soluble IL-1R AcM polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in Figure 1 [SEQ ID NO:2], or a peptide or polypeptide comprising a portion of the above polypeptides. The terms "peptide" and "oligopeptide" are considered synonymous (as is commonly recognized) and each term can be used interchangeably as the context requires to indicate a chain of at least to amino acids coupled by peptidyl linkages. The word "polypeptide" is used herein for chains containing more than ten amino acid residues. All oligopeptide and polypeptide formulas or sequences herein are written from left to right and in the direction from amino terminus to carboxy terminus.

It will be recognized in the art that some amino acid sequences of the soluble IL-1R AcM polypeptide can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity. In general, it is possible to replace residues which form the tertiary structure, provided that residues performing a similar function are used. In other instances, the type of residue may be completely unimportant if the alteration occurs at a non-critical region of the protein.

Thus, the invention further includes variations of the soluble IL-1R AcM polypeptide which show substantial soluble IL-1R AcM polypeptide activity or which include regions of soluble IL-1R AcM protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U., *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990).

Thus, the fragment, derivative or analog of the polypeptide of SEQ ID NO:2, or that encoded by the deposited cDNA, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the soluble IL-1R AcM protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard *et al.*, *Clin. Exp. Immunol.* 2:331-340 (1967); Robbins *et al.*, *Diabetes* 36:838-845 (1987); Cleland *et al.* *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993)).

The replacement of amino acids can also change the selectivity of binding to or interaction with cell surface receptors. Ostade *et al.*, *Nature* 361:266-268 (1993) describes certain mutations resulting in selective binding of TNF- $\alpha$  to only one of the two known types of TNF receptors. Thus, the soluble IL-1R AcM of the present invention may include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 1).

TABLE 1. Conservative Amino Acid Substitutions.

Aromatic	Phenylalanine Tryptophan Tyrosine
Hydrophobic	Leucine Isoleucine Valine
Polar	Glutamine Asparagine
Basic	Arginine Lysine Histidine
Acidic	Aspartic Acid Glutamic Acid
Small	Alanine Serine Threonine Methionine Glycine

Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of substitutions for any given soluble IL-1R AcM polypeptide will not be more than 50, 40, 30, 25, 20, 15, 10, 5 or 3.

Amino acids in the soluble IL-1R AcM protein of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as receptor binding or *in vitro*, or *in vitro* proliferative activity. Sites that are critical for ligand-receptor binding or interaction can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith *et al.*, *J. Mol. Biol.* 224:899-904 (1992) and de Vos *et al.* *Science* 255:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide" is intended a polypeptide removed from

its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell. For example,  
5 a recombinantly produced version of the soluble IL-1R AcM polypeptide can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988).

The polypeptides of the present invention include the polypeptide encoded by the deposited cDNA including the leader, the mature polypeptide encoded by  
10 the deposited the cDNA minus the leader (i.e., the mature protein), a polypeptide comprising amino acids about - 17 to about 339 in SEQ ID NO:2; a polypeptide comprising amino acids about - 16 to about 339 in SEQ ID NO:2; a polypeptide comprising amino acids about 1 to about 339 in SEQ ID NO:2; as well as polypeptides which are at least 80% identical, more preferably at least 90% or  
15 95% identical, still more preferably at least 96%, 97%, 98% or 99% identical to those described above and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a soluble IL-1R AcM  
20 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of the soluble IL-1R AcM polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a  
25 reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the  
30 reference amino acid sequence or anywhere between those terminal positions,

interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in Figure 1 [SEQ ID NO:2] or to the amino acid sequence encoded by deposited cDNA clone can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

The polypeptide of the present invention could be used as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

As described in detail below, the polypeptides of the present invention can also be used to raise polyclonal and monoclonal antibodies, which are useful in assays for detecting soluble IL-1R AcM protein expression as described below or as agonists and antagonists capable of enhancing or inhibiting soluble IL-1R AcM protein function. Further, such polypeptides can be used in the yeast two-hybrid system to "capture" soluble IL-1R AcM protein binding proteins which are also candidate agonist and antagonist according to the present invention. The yeast two hybrid system is described in Fields and Song, *Nature* 340:245-246 (1989).

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of



a protein that elicits an antibody response when the whole protein is the immunogen. These immunogenic epitopes are believed to be confined to a few loci on the molecule. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen *et al.*, *Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R.A. (1983) Antibodies that react with predetermined sites on proteins. *Science* 219:660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson *et al.*, *Cell* 37:767-778 (1984) at 777.

Antigenic epitope-bearing peptides and polypeptides of the invention designed according to the above guidelines preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

Non-limiting examples of antigenic polypeptides or peptides that can be used to generate soluble IL-1R AcM-specific antibodies include the following: a polypeptide comprising amino acid residues from about 6 to about 15 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 57 to about 66

in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 70 to about 79 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 106 to about 112 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 115 to about 124 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 129 to about 135 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 158 to about 172 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 180 to about 187 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 207 to about 215 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 231 to about 244 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 247 to about 255 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 268 to about 276 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 285 to about 295 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 303 to about 310 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 319 to about 330 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from about 333 to about 339 in SEQ ID NO:2.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. Houghten, R. A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. USA* 82:5131-5135. This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten *et al.* (1986).

As one of skill in the art will appreciate, soluble IL-1R AcM polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or

light chains of mammalian immunoglobulins (EPA 394,827; Traunecker *et al.*,  
*Nature* 331:84- 86 (1988)). Fusion proteins that have a disulfide-linked dimeric  
structure due to the IgG part can also be more efficient in binding and neutralizing  
other molecules than the monomeric soluble IL-1R AcM protein or protein  
fragment alone (Fountoulakis *et al.*, *J. Biochem* 270:3958-3964 (1995)).

### ***Screening Applications of Soluble IL-1R AcM***

The present inventors believe that soluble IL-1R AcM is involved in IL-1  
activity and that it forms a complex with the Type I IL-1R allowing IL-1 $\beta$  to bind  
with higher affinity than to the Type I IL-1R alone. Thus, the presence or absence  
of the accessory molecule in different cell lines may determine whether the low  
or the high affinity site in the Type I IL-1R is formed. The low affinity site  
corresponds to the Type I IL-1R alone, while the higher affinity site represents a  
complex of the Type I IL-1R with the IL-1R AcM. Given that IL-1 has many  
diverse effects on immunologic and inflammatory process and that IL-1 elicits a  
wide variety of effects in hematic and nonhematic cells, it would be of interest  
to identify agonist and antagonist for IL-1 activity. IL-1R AcM may to be  
necessary for IL-1 signal transduction events and may to be required for the  
formation of a high affinity IL-1R binding state. Stable cell lines established by  
simultaneous cotransfection of two expression vectors such that both IL-1R and  
IL-1R AcM are expressed at a 1:10 ratio of molecules/cell and control cell lines  
which express only IL-1R or IL-1R AcM could be used in screening assays to  
identify potential agonists and antagonists for IL-1. Thus, the present invention  
further provides a screening method of identifying IL-1 receptor agonists, which  
involves: (a) providing a host cell containing recombinant genes which express  
a polypeptide comprising a type I IL-1 receptor and a polypeptide comprising IL-  
1R AcM or a IL-1R AcM fragment, wherein IL-1R and IL-1R AcM or an IL-1R  
AcM fragment form a complex; (b) administering a candidate agonist to said cell;  
and (c) determining the binding affinity of said complex for said candidate  
agonist relative to the binding of said complex for IL-1.

To characterize candidate IL-1R agonist binding to IL-1R, each of the cell lines are analyzed by equilibrium binding with  $^{125}\text{I}$ -labeled IL-1 $\beta$ . Equilibrium binding of  $^{125}\text{I}$ -labeled IL-1 $\beta$  to the cells can be performed as described by Mizel, *et al. J. Immunol.* 138: 2906-2912 (1987) and Greenfeder *et al., J. Biol. Chem.* 270:13757-13765 (1995). The activity of the candidate agonist, can be examined relative to the binding of IL-1 $\beta$  to a CHO-IR/AcM cell line (as described, *supra*). It is desirable for this cell line to express an excess amount of IL-1R AcM protein relative to IL-1R. Cell lines bearing only IL-1R AcM protein will not bind IL-1 $\beta$  and cell lines bearing only IL-R bind IL-1 $\beta$  with low affinity (i.e., on the order of  $K_D$  1.0-3.3nM). A CHO-IR/AcM cell line, bearing both the IL-1R and IL-1R AcM results in the IL-1R having a higher affinity binding site (i.e., on the order of  $K_D$  0.02-0.8 nM). Thus, one can monitor the relative binding affinity of the candidate agonist relative to the IL-1 $\beta$ . The presence of the IL-1R AcM protein of the present invention in the cell line used for agonist screening ensures that the IL-1R is in a high affinity binding state.

In a further aspect, the invention provides a screening method of identifying an IL-1 signal transduction antagonist. The results of Greenfeder, *et al. (J. Biol. Chem.* 270: 13757-13765 (1995)) suggest that the antagonist IL-1ra prevents or disrupts formation of a complex between muIL-1R and muIL-1R AcP. Thus, the screening method for identifying other antagonist of signal transduction which involves: (a) providing a host cell containing recombinant genes which express a polypeptide comprising a type I IL-1 receptor and a polypeptide comprising IL-1R AcM or an IL-1R AcM fragment, wherein IL-1R and IL-1R AcM or IL-1R and the IL-1R AcM fragment form a complex; (b) administering a candidate antagonist to said cell; and (c) determining the whether said candidate antagonist disrupts or prevents formation of a complex between IL-1R and IL-1R AcM or IL-1R and the IL-1R AcM fragment.

In this antagonist screening assay, one can measure the formation of complex between IL-1R and IL-1R AcM by immunoprecipitating labeled protein complexes cross-linked to IL-1 $\beta$ , IL-1ra or the candidate antagonist. Using an anti-type I IL-1R mAb or an anti-IL-1R AcM mAb, a protein complex of > 200

kDa range will be observed for the cross linking reaction when IL-1 $\beta$  is used as a ligand indicating formation of an IL-1 binding complex. In addition, these antibodies will immunoprecipitate labeled IL1 $\beta$ . However, when labeled IL-1ra or a candidate antagonist is used as ligand, the anti-IL-1R AcM mAb will not immunoprecipitate the labeled agonist. Rather, the anti-IL-1R AcM antibody will only precipitate an IL-1ra or a candidate antagonist complex in the 100-120 kDa range indicating that the IL-1R and IL-1R AcM have not formed a complex.

#### **Alternative screening assay**

The present invention further provides a screening method for identifying IL-1 receptor agonists and antagonists, which involves: (a) providing a polypeptide comprising a type I IL-1 receptor and a polypeptide comprising IL-1R AcM or IL-1R AcM fragment, wherein IL-1R and IL-1R AcM or IL-1R and the IL-1R AcM fragment form a complex; (b) providing a candidate compound; (c) providing a polypeptide comprising IL-1 or a functional IL-1 fragment; and (d) determining the binding affinity of said complex for IL-1 whereby an increased binding affinity of said complex for IL-1 in the presence of said compound is indicative that said compound is an agonist for IL-1 signal transduction and a decreased binding affinity of said complex for IL-1 in the presence of said compound is indicative that said compound is an antagonist of IL-1 signal transduction.

#### **Candidate Agonists and Antagonists**

Candidate antagonists and agonist according to the present invention include IL-1ra, polypeptides and antibodies that either enhance or inhibit formation of the IL-1R IL-1R AcM complex. For example, an antibody that inhibits the murine IL-1R-IL-1R accessory protein complex is described in Greenfeder, *et al. J. Biol. Chem.* 270: 13757-13765 (1995). In addition, antibodies directed against murine type I IL-1 receptors which block IL-1 activity are described in Chizzonite *et al. Proc. Natl Acad Sci USA* 86:8029 (1989); Lewis *et al. Eur. J. Immunol.* 20:207 (1990); Dinarello, *Int. J. Clin. Lab. Res.* 24:61-79

(1994). In addition, a point mutation in IL-1ra converts IL-1ra from an antagonist to a partial agonist of IL-1 activity in Ju *et al. Proc. Natl. Acad. Sci USA* 88:2658-2662 (1991). Others have developed recombinant IL-1 mutants with altered activities (Dinarello *Blood* 77:1627-1652; Gehrke *et al. J. Biol. Chem.* 265:5922-5925 (1990)). Thus, methods are known in the art for developing candidate IL-1R agonists and antagonists for screening in the present invention.

Methods for determining agonist or antagonist activity are known in the art (Ju *et al. Proc. Natl. Acad. Sci USA* 88:2658-2662 (1991); Dinarello *Blood* 77:1627-1652; Gehrke *et al. J. Biol. Chem.* 265:5922-5925 (1990)). Methods for determining whether a candidate agonist or antagonist enhances or interferes with the formation of the IL-1 receptor complex between IL-1R and IL-1R AcM is disclosed in Greenfeder *et al., J. Biol. Chem.* 270:13757-13765 (1995).

Administration of the candidate agonist or antagonist can be exogenous or endogenous and the candidate agonist or antagonist can be obtained from natural or recombinant sources. In addition, the screening method further provides for host cells containing recombinant genes expressing IL-1R and IL-1R AcM, as described above.

By "a host cell containing recombinant genes" is intended host cells which one or more of the recombinant constructs described herein have been introduced or a progeny of such host cells.

In addition, the invention provides antibodies directed to this accessory molecule which inhibit the interaction of IL-1R with IL-1R AcM thereby modulating IL-1 response of the cells. These antibodies could be useful for immunoprecipitating cross-linked complexes for the antagonist screening assay as well as showing that the stable cell lines are in fact expressing the type I IL-1R and IL-1R AcM proteins. Methods for obtaining these antibodies are set forth below.

Soluble IL-1R AcM protein-specific antibodies for use in the present invention can be raised against the intact soluble IL-1R AcM protein or an antigenic polypeptide portion thereof, which may presented together with a

carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody portions (such as, for example, Fab and F(ab')<sub>2</sub> portions) which are capable of specifically binding to soluble IL-1R AcM protein. Fab and F(ab')<sub>2</sub> portions lack the Fc portion of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl *et al.*, *J. Nucl. Med.* 24:316-325 (1983)). Thus, these portions are preferred.

The antibodies of the present invention may be prepared by any of a variety of methods. For example, cells expressing the soluble IL-1R AcM protein or an antigenic portion thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of soluble IL-1R AcM protein is prepared and purified as described above to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or soluble IL-1R AcM protein binding portions thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler *et al.*, *Nature* 256:495 (1975); Kohler *et al.*, *Eur. J. Immunol.* 6:511 (1976); Kohler *et al.*, *Eur. J. Immunol.* 6:292 (1976); Hammerling *et al.*, In: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, such procedures involve immunizing an animal (preferably a mouse) with a soluble IL-1R AcM protein antigen or, more preferably, with a soluble IL-1R AcM protein-expressing cell. Suitable cells can be recognized by their capacity to bind soluble IL-1R AcM protein antibody. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 µg/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml

of streptomycin. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP<sub>2</sub>O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands *et al.* (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the soluble IL-1R AcM antigen.

Alternatively, additional antibodies capable of binding to the soluble IL-1R AcM protein antigen may be produced in a two-step procedure through the use of anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, soluble IL-1R AcM protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the soluble IL-1R AcM protein-specific antibody can be blocked by the soluble IL-1R AcM protein antigen. Such antibodies comprise anti-idiotypic antibodies to the soluble IL-1R AcM protein-specific antibody and can be used to immunize an animal to induce formation of further soluble IL-1R AcM protein-specific antibodies.

It will be appreciated that Fab and F(ab')<sub>2</sub> and other portions of the antibodies of the present invention may be used according to the methods disclosed herein. Such portions are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab portions) or pepsin (to produce F(ab')<sub>2</sub> portions). Alternatively, soluble IL-1R AcM protein-binding portions can be produced through the application of recombinant DNA technology or through synthetic chemistry.



Where *in vivo* imaging is used to detect enhanced levels of soluble IL-1R AcM protein for diagnosis in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. See, for review, Morrison, *Science* 229:1202 (1985); Oi *et al.*, *BioTechniques* 4:214 (1986); Cabilly *et al.*, U.S. Patent No. 4,816,567; Taniguchi *et al.*, EP 171496; Morrison *et al.*, EP 173494; Neuberger *et al.*, WO 8601533; Robinson *et al.*, WO 8702671; Boulianne *et al.*, *Nature* 312:643 (1984); Neuberger *et al.*, *Nature* 314:268 (1985).

Further suitable labels for the soluble IL-1R AcM protein-specific antibodies of the present invention are provided below. Examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast-alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholine esterase.

Examples of suitable radioisotopic labels include  $^3\text{H}$ ,  $^{111}\text{In}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ ,  $^{51}\text{Cr}$ ,  $^{57}\text{To}$ ,  $^{58}\text{Co}$ ,  $^{59}\text{Fe}$ ,  $^{75}\text{Se}$ ,  $^{152}\text{Eu}$ ,  $^{90}\text{Y}$ ,  $^{67}\text{Cu}$ ,  $^{217}\text{Ci}$ ,  $^{211}\text{At}$ ,  $^{212}\text{Pb}$ ,  $^{47}\text{Sc}$ ,  $^{109}\text{Pd}$ , etc.  $^{111}\text{In}$  is a preferred isotope where *in vivo* imaging is used since it avoids the problem of dehalogenation of the  $^{125}\text{I}$  or  $^{131}\text{I}$ -labeled monoclonal antibody by the liver. In addition, this radionuclide has a more favorable gamma emission energy for imaging (Perkins *et al.*, *Eur. J. Nucl. Med.* 10:296-301 (1985); Carasquillo *et al.*, *J. Nucl. Med.* 28:281-287 (1987)). For example,  $^{111}\text{In}$  coupled to monoclonal antibodies with 1-(P-isothiocyanatobenzyl)-DPTA has shown little uptake in non-tumorous tissues, particularly the liver, and therefore enhances specificity of tumor localization (Esteban *et al.*, *J. Nucl. Med.* 28:861-870 (1987)).

Examples of suitable non-radioactive isotopic labels include  $^{157}\text{Gd}$ ,  $^{55}\text{Mn}$ ,  $^{162}\text{Dy}$ ,  $^{52}\text{Tr}$ , and  $^{56}\text{Fe}$ .

Examples of suitable fluorescent labels include an  $^{152}\text{Eu}$  label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, an o-phthaldehyde label, and a fluorescamine label.

5           Examples of suitable toxin labels include diphtheria toxin, ricin, and cholera toxin.

          Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label,  
10           and an aequorin label.

          Examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, and Fe.

          Typical techniques for binding the above-described labels to antibodies are provided by Kennedy *et al.* (*Clin. Chim. Acta* 70:1-31 (1976)), and Schurs *et al.* (*Clin. Chim. Acta* 81:1-40 (1977)). Coupling techniques mentioned in the  
15           latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

### ***Chromosome Assays***

20           The nucleic acid molecules of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data  
25           (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a soluble IL-1R AcM protein gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for  
5 *in situ* chromosome mapping using well known techniques for this purpose. Typically, in accordance with routine procedures for chromosome mapping, some trial and error may be necessary to identify a genomic probe that gives a good *in situ* hybridization signal.

In addition, in some cases, sequences can be mapped to chromosomes by  
10 preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids  
15 containing the human gene corresponding to the primer will yield an amplified portion.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of  
20 portions from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization ("FISH") of a cDNA clone to a  
25 metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma *et al.*, *Human Chromosomes: A Manual Of Basic Techniques*, Pergamon Press, New York  
30 (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance In Man*, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. This assumes 1 megabase mapping resolution and one gene per 20 kb.

#### ***Therapeutic Uses of Soluble IL-1R AcM***

IL-1 has an important role in modulating the proliferation, maturation, and functional activation of hematopoietic cells, including lymphoid and nonlymphoid cells. IL-1 may have an important function in the regulation liver metabolism and be responsible for some of the marked changes in hepatic protein synthesis that occur in the acute phase response to inflammation or tissue injury.

IL-1 is also involved in the regulation of bone remodeling, may be involved in the pathogenesis of chronic inflammatory joint diseases, such as rheumatoid arthritis, osteoarthritis, and may also play a role in the mechanisms of articular cartilage destruction that occurs in degradative arthropathies. Human IL-1 is capable of increasing collagen protein and mRNA levels in cultured normal human dermal fibroblasts, thus, IL-1 may have a role in the early stages of scleroderma and other fibrotic diseases. IL-1 is capable of inducing a proliferative response in fibroblasts. In addition, IL-1 may have important effects

on vascular cells, including endothelial cells and vascular smooth muscle cells. IL-1 may be involved in the pathogenesis of certain skin diseases, including chronic diseases such as psoriasis and epithelial fungus infections. IL-1 may have important effects on the functions of the hypothalamus-pituitary axis and thyroid gland. IL-1 has an important role in the regulation of insulin secretion by  $\beta$  cells in the pancreatic islets of Langerhans. Finally, IL-1 has important effects on the gonads and may play a role in the physiology of neural tissues. (Reviewed in Pimentel, *Handbook of Growth Factors: Volume III Hematopoietic Growth Factors and Cytokines*, pp. 35-53, CRC Press, Boca Raton, FL 1994).

### ***Antibody Therapy***

In view of the wide range of roles that IL-1 plays in physiologic and pathologic processes, regulating the action of IL-1 by abrogating signal transduction from the IL-1 binding complex is expected to be useful for therapeutic purposes. For example, recombinant IL-1ra blocks the activity of exogenously administered IL-1 in a variety of animal models. When rabbits or baboons are injected with IL-1 they develop hypertension which is prevented by a prior injection of IL-1ra (Ohlsson *et al. Nature* 348: 550 (1990); Fischer *et al. Am. J. Physiol.* 261: R442 (1991)). In animal studies, IL-1R blockade significantly reduces the severity of diseases, including those associated with infections, inflammation and metabolic disturbances (Arend, W.P. *J. Clin. Invest* 88:1445 (1991); Dinarello *et al. Immunol. Today* 12: 404 (1991)). In Table 1 of Dinarello, *Int. J. Clin. Lab. Res.* 24:61-79 (1994) (which is incorporated herein by reference) different models are listed wherein a specific reduction in IL-1R activity has been employed to reduce the disease severity.

In addition, studies with human subjects have also demonstrated that blockade of IL-1R is effective where the severity of disease is high. For example, dramatic results have been seen in patients with septic shock. In clinical trials there was a statistically significant reduction in mortality. (Dinarello, *Int. J. Clin. Lab. Res.* 24:61-79 (1994))

In another clinical trial, rheumatoid arthritis patients treated with IL-1ra, in addition to other non-steroidal anti-inflammatory drugs, had a significant reduction in the number and severity of painful and swollen joints. These results demonstrate an improvement in the clinical disease of these patients. (Dinarello, *Int. J. Clin. Lab. Res.* 24:61-79 (1994))

Similarly, antibodies produced to the murine type I IL-1R have been used to block IL-1 effects *in vitro* and *in vivo*. (Chizzonite *et al. Proc. Natl Acad Sci USA* 86:8029 (1989); Lewis *et al. Eur. J. Immunol.* 20:207 (1990)). For example, in animal models of infection and inflammation, administration of anti-IL-1R antibodies have reduced disease severity (Dinarello, *Int. J. Clin. Lab. Res.* 24:61-79 (1994)). The advantage of the anti-IL-1R antibodies is that they block IL-1 effects for several hours to days, whereas IL-1ra blood levels need to be sustained at sufficiently high levels to block IL-1 effects (Dinarello, *Int. J. Clin. Lab. Res.* 24:61-79 (1994)).

Given that IL-1R AcM is involved in IL-1 signal transduction, antibodies directed against IL-1R AcM are expected to behave as agonists or antagonist of IL-1 activity. For example, an antibody directed against the murine IL-1R accessory protein blocked the binding of IL-1 $\beta$  to murine type I IL-1R (Greenfeder *et al. J. Biol. Chem.* 270: 13757-13765 (1995)). Thus, antibodies directed against the IL-1R AcM of the present invention that abrogate IL-1 activity can be used therapeutically to reduce the severity of diseases associated with IL-1.

Thus, the present invention is further directed antibody-based therapies which involve administering an anti-IL-1R AcM antibody to a mammalian, preferably human, patient for treating one or more of above-described disorders. Methods for producing anti-IL-1R AcM polyclonal and monoclonal antibodies are described in detail above. Such antibodies can may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding IL-1R AcM locally or systemically

in the body or by direct cytotoxicity of the antibody, *e.g.*, as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The pharmaceutical compositions of the present invention may be administered by any means that achieve their intended purpose. Amounts and regimens for the administration of antibodies, their fragments or derivatives can be determined readily by those with ordinary skill in the clinical art of treating colon cancer and related disease.

For example, administration may be by parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

Compositions within the scope of this invention include all compositions wherein the antibody, fragment or derivative is contained in an amount effective to achieve its intended purpose. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. The effective dose is a function of the individual chimeric or monoclonal antibody, the presence and nature of a conjugated therapeutic agent (see below), the patient and his clinical status, and can vary from about 10  $\mu\text{g/kg}$  body weight to about 5000 mg/kg body weight. The preferred dosages comprise 0.1 to 500 mg/kg body wt.

In addition to the pharmacologically active compounds, the new pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically.

Preferably, the preparations, contain from about 0.01 to 99 percent, preferably from about 20 to 75 percent of active compound(s), together with the excipient.

Similarly, preparations of an IL-1R AcM antibody or fragment of the present invention for parenteral administration, such as in detectably labeled form for imaging or in a free or conjugated form for therapy, include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media, parenteral vehicles including sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. See, generally, *Remington's Pharmaceutical Science*, 16th ed., Mack Publishing Co., Easton, PA, 1980.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating a subject having or developing IL-1R AcM related disorders as described herein. Such treatment comprises parenterally administering a single or multiple doses of the antibody, fragment or derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hemopoietic growth factors, etc., which serve to increase the number or activity of effector cells which interact with the antibodies.

Since it appear to be necessary to block nearly all IL-1R's to block IL-1 activity, it is preferred to use high affinity and/or potent *in vivo* IL-1R AcM-inhibiting and/or neutralizing antibodies, fragments or regions thereof, for both IL-1R AcM immunoassays and therapy of IL-1 related disorders. Such antibodies, fragments, or regions, will preferably have an affinity for human IL-1R AcM, expressed as  $K_a$ , of at least  $10^8 \text{ M}^{-1}$ , more preferably, at least  $10^9 \text{ M}^{-1}$ ,



such as  $5 \times 10^8 \text{ M}^{-1}$ ,  $8 \times 10^8 \text{ M}^{-1}$ ,  $2 \times 10^9 \text{ M}^{-1}$ ,  $4 \times 10^9 \text{ M}^{-1}$ ,  $6 \times 10^9 \text{ M}^{-1}$ ,  $8 \times 10^9 \text{ M}^{-1}$ .

Preferred for human therapeutic use are high affinity murine and murine/human or human/human chimeric antibodies, and fragments, regions and derivatives having potent *in vivo* IL-1-inhibiting and/or neutralizing activity, according to the present invention, *e.g.*, that block IL-1-induced IL-6 secretion, and mitogenic activity, *in vivo*, *in situ*, and *in vitro*.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

### ***Examples***

#### ***Example 1: Expression and Purification of IL-1R AcM in E. coli***

The DNA sequence encoding the mature soluble IL-1R AcM protein in the deposited cDNA clone is amplified using PCR oligonucleotide primers specific to the amino acid carboxyl terminal sequence of the soluble IL-1R AcM protein and to vector sequences 3' to the gene. Additional nucleotides containing restriction sites BamHI and SalI to facilitate cloning are added to the 5' and 3' sequences, respectively.

The 5' oligonucleotide primer has the sequence 5' GGATCCATGACACTTCTGTGGTGTG 3' (SEQ ID NO:23) containing the underlined BamHI restriction site, followed by 16 nucleotides complementary to bp 1834-1853 of the antisense strand of the IL-1R

AcM protein coding sequence set out in Figure 1 (SEQ ID NO:1).

The 3' primer has the sequence 5' GTCGACTCACTGACCGCATCT 3' (SEQ ID NO:24) containing the underlined SalI restriction site, followed by 15 nucleotides complementary to bp 1056-1071 of the sense strand of the IL-1R

AcM protein coding sequence set out in Figure 1 (SEQ ID NO:1), and a stop codon.

The restriction sites are convenient to restriction enzyme sites in the bacterial expression vector pQE-9, which is used for bacterial expression in these examples. (Qiagen, Chatsworth, CA, 91311).

The amplified IL-1R AcM protein DNA and the vector pQE-9 are both digested with BamHI and SalI and the digested DNAs are subsequently ligated together. Insertion of the IL-1R AcM protein DNA into the pQE-9 restricted vector places the IL-1R AcM protein coding region downstream of and operably linked to the vector's promoter and in-frame with an initiating AUG appropriately positioned for translation of IL-1R AcM protein.

The ligation mixture is transformed into competent *E. coli* cells using standard procedures. Such procedures are described, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). *E. coli* strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses lac repressor and confers kanamycin resistance ("Kanr"), is used in carrying out the illustrative example described here. This strain, which is only one of many that are suitable for expressing IL-1R AcM protein, is available commercially from Qiagen.

Transformants are identified by their ability to grow on LB plates in the presence of ampicillin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA is confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 µg/ml) and kanamycin (25 µg/ml).

The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:100 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-B-D-thiogalactopyranoside ("IPTG") are then added to a final concentration of 1 mM to induce transcription from lac repressor sensitive promoters, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells are then harvested by

centrifugation and disrupted, by standard methods. Inclusion bodies are purified from the disrupted cells using routine collection techniques, and protein are solubilized from the inclusion bodies into 8M urea. The 8M urea solution containing the solubilized protein is passed over a PD-10 column in 2X phosphate buffered saline ("PBS"), thereby removing the urea, exchanging the buffer and refolding the protein. The protein is purified by a further step of chromatography to remove endotoxin. Then, it is sterile filtered. The sterile filtered protein preparation is stored in 2X PBS at a concentration of 95 micrograms per mL.

Analysis of the preparation by standard methods of polyacrylamide gel electrophoresis reveals that the preparation contains about 95% monomer IL-1R AcM protein having the expected molecular weight of approximately 42 kDa.

### ***Example 2: Cloning and Expression in Mammalian Cells***

Most of the vectors used for the transient expression of the IL-R AcM protein gene sequence in mammalian cells should carry the SV40 origin of replication. This allows the replication of the vector to high copy numbers in cells (e.g., COS cells) which express the T antigen required for the initiation of viral DNA synthesis. Any other mammalian cell line can also be utilized for this purpose.

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However, cellular signals can also be used (e.g., human actin promoter). Suitable

expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include, human Hela, 283, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, African green monkey cells, quail QC1-3 cells, mouse L cells and Chinese hamster ovary cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) is a useful marker to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy *et al.*, *Biochem J.* 227:277-279 (1991); Bebbington *et al.*, *Bio/Technology* 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) cells are often used for the production of proteins.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen *et al.*, *Molecular and Cellular Biology*, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart *et al.*, *Cell* 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

**Example 2(a): Cloning and Expression in COS Cells**

The expression plasmid, pIL-1R AcM HA, is made by cloning a cDNA encoding IL-R AcM into the expression vector pcDNAI/Amp (which can be obtained from Invitrogen, Inc.).

The expression vector pcDNAI/amp contains: (1) an *E.coli* origin of replication effective for propagation in *E. coli* and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eukaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron, and a polyadenylation signal arranged so that a cDNA conveniently can be placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker.

A DNA fragment encoding the IL-1R AcM protein and an HA tag fused in frame to its 3' end is cloned into the polylinker region of the vector so that recombinant protein expression is directed by the CMV promoter. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein described by Wilson *et al.*, *Cell* 37: 767 (1984). The fusion of the HA tag to the target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is as follows. The IL-1R AcM cDNA of the deposited clone is amplified using primers that contain convenient restriction sites, much as described above regarding the construction of expression vectors for expression of IL-R AcM in *E. coli*. To facilitate detection, purification and characterization of the expressed IL-1R AcM, one of the primers contains a hemagglutinin tag ("HA tag") as described above.

Suitable primers include the following, which are used in this example. The 5' primer, containing the underlined BamHI site, an AUG start codon and 5 codons of the 5' coding region has the following sequence:

5' GGATCCATCCGCCATCATGACACTTCTGTGGTGTG 3'  
(SEQ ID NO:25).

The 3' primer, containing the underlined XbaI site, a stop codon, 9 codons thereafter forming the hemagglutinin HA tag, and 12 bp of 3' coding sequence (at the 3' end) has the following sequence:

5' TCTAGAAAAGCGTAGTCTGGGACGTCGTATGGGTAC  
TGACCGCATCT 3' (SEQ ID NO:26).

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with BamHI and XbaI and then ligated. The ligation mixture is transformed into *E. coli* strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis and gel sizing for the presence of the IL-1R AcM-encoding fragment.

For expression of recombinant IL-1R AcM, COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook *et al.*, Molecular Cloning: a Laboratory Manual, Cold Spring Laboratory Press, Cold Spring Harbor, New York (1989). Cells are incubated under conditions for expression of IL-1R AcM by the vector.

Expression of the IL-1R AcM HA fusion protein is detected by radiolabelling and immunoprecipitation, using methods described in, for example Harlow *et al.*, Antibodies: A Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing <sup>35</sup>S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and the lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson *et al.* cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated proteins then are analyzed by SDS-PAGE gels and autoradiography. An

expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

***Example 2(b): Cloning and Expression in CHO Cells***

The vector pC1 is used for the expression of IL-1R AcM protein. Plasmid pC1 is a derivative of the plasmid pSV2-dhfr [ATCC Accession No. 37146]. Both plasmids contain the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt, F.W., Kellems, R.M., Bertino, J.R., and Schimke, R.T., 1978, J. Biol. Chem. 253:1357-1370, Hamlin, J.L. and Ma, C. 1990, Biochem. et Biophys. Acta, 1097:107-143, Page, M.J. and Sydenham, M.A. 1991, Biotechnology Vol. 9:64-68). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene it is usually co-amplified and over-expressed. It is state of the art to develop cell lines carrying more than 1,000 copies of the genes. Subsequently, when the methotrexate is withdrawn, cell lines contain the amplified gene integrated into the chromosome(s).

Plasmid pC1 contains for the expression of the gene of interest a strong promoter of the long terminal repeat (LTR) of the Rouse Sarcoma Virus (Cullen, *et al.*, Molecular and Cellular Biology, March 1985:438-4470) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart *et al.*, *Cell* 41:521-530, 1985). Downstream of the promoter are the following single restriction enzyme cleavage sites that allow the integration of the genes: BamHI, PvuII, and NruI. Behind these cloning sites the plasmid contains translational stop codons in all three reading frames

followed by the 3' intron and the polyadenylation site of the rat preproinsulin gene. Other high efficient promoters can also be used for the expression, e.g., the human  $\beta$ -actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLV. For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well.

Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC1 is digested with the restriction enzyme BamHI and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding IL-1R AcM, ATCC 97666, is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

The 5' primer has the sequence 5'GACTGGATCCGCCATCATGACACTTCTGTGGTGTG 3' (SEQ ID NO:27) containing the underlined BamHI restriction enzyme site followed by 19 bases of the sequence of IL-1R AcM of Figure 1 (SEQ ID NO:1). Inserted into an expression vector, as described below, the 5' end of the amplified fragment encoding human IL-1R AcM provides an efficient signal peptide. An efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., J. Mol. Biol. 196:947-950 (1987) is appropriately located in the vector portion of the construct.

The 3' primer has the sequence 5' GAATTCCTCACTGACCGCATCT 3' (SEQ ID NO:28) containing the EcoRI restriction followed by nucleotides complementary to the last 15 nucleotides of the IL-1R AcM coding sequence set out in Figure 1 (SEQ ID NO:1), including the stop codon.



The amplified fragments are isolated from a 1% agarose gel as described above and then digested with the endonucleases BamHI and EcoRI and then purified again on a 1% agarose gel.

The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 cells are then transformed and bacteria identified that contained the plasmid pC1 inserted in the correct orientation using the restriction enzyme BamHI. The sequence of the inserted gene is confirmed by DNA sequencing.

#### Transfection of CHO-DHFR-cells

Chinese hamster ovary cells lacking an active DHFR enzyme are used for transfection. 5 µg of the expression plasmid C1 are cotransfected with 0.5 µg of the plasmid pSVneo using the lipofecting method (Felgner *et al.*, *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the gene neo from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) and cultivated from 10-14 days. After this period, single clones are trypsinized and then seeded in 6-well petri dishes using different concentrations of methotrexate (25 nM, 50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 µM, 2 µM, 5 µM, 10 mM, 20mM). The same procedure is repeated until clones grow at a concentration of 100 µM.

The expression of the desired gene product is analyzed by Western blot analysis and SDS-PAGE or by reverse phase HPLC analysis.

**Example 3: Cloning and expression of the Soluble IL-1R AcM protein in a baculovirus expression system**

The cDNA sequence encoding the soluble IL-1R AcM protein in the deposited clone was amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene:

The 5' primer has the sequence 5' GACTGGATCCGCCATCATGACACTTCTGTGGTGTG 3' (SEQ ID NO:29) containing the underlined BamHI restriction enzyme site followed by 19 bases (bp 1834-1853) complementary to the antisense strand of the soluble IL-1R AcM protein coding sequence of Figure 1 (SEQ ID NO:1). Inserted into an expression vector, as described below, the 5' end of the amplified fragment encoding soluble IL-1R AcM protein receptor provides an efficient signal peptide. An efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol.* 196:947-950 (1987), may be located, as appropriate, in the vector portion of the construct.

For the full length gene, the 3' primer has the full length sequence 5' GAC TGG TAC CCA TAG AAA TCA TGT GTA TAC C 3' (SEQ ID NO:30), containing the underlined Asp718 restriction followed by 25 nucleotides complementary to bp 2049-2070 of the sense strand of the soluble IL-1R AcM protein set out in Figure 1 [SEQ ID NO:1], and a stop codon.

The amplified fragment was isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with BamHI and Asp718 and again is purified on a 1% agarose gel. This fragment is designated herein "F2".

The vector pA2 is used to express the soluble IL-1R AcM protein in the baculovirus expression system, using standard methods, such as those described in Summers *et al.*, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures*, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites. For an easy selection of recombinant virus the beta-

galactosidase gene from *E. coli* is inserted in the same orientation as the polyhedrin promoter and is followed by the polyadenylation signal of the polyhedrin gene. The polyhedrin sequences are flanked at both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that express the cloned polynucleotide.

Many other baculovirus vectors could be used in place of pA2, such as pAc373, pVL941 and pAcIM1 provided, as those of skill readily will appreciate, that construction provides appropriately located signals for transcription, translation, trafficking and the like, such as an in-frame AUG and a signal peptide, as required. Such vectors are described, for example, in Luckow *et al.*, *Virology* 170:31-39 (1989). Suitable vectors will be readily apparent to the skilled artisan.

The plasmid was digested with the restriction enzymes BamHI and Asp718 and then was dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA was then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V2".

Fragment F2 and the dephosphorylated plasmid V2 were ligated together with T4 DNA ligase. *E. coli* HB 101 cells were transformed with ligation mix and spread on culture plates. Bacteria were identified that contain the plasmid with the human soluble IL-1R AcM protein gene by digesting DNA from individual colonies using BamHI and Asp718 and then analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment was confirmed by DNA sequencing. This plasmid is designated herein as pA2HG16302.

5 µg of plasmid pA2HG16302 was co-transfected with 1.0 µg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner *et al.*, *Proc. Natl. Acad. Sci. USA* 84:7413-7417 (1987). 1 µg of BaculoGold™ virus DNA and 5 µg of the plasmid pA2HG16302 were mixed in a sterile well of a microliter plate containing 50 µl of serum free Grace's medium

(Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 µl Lipofectin plus 90 µl Grace's medium were added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture was added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate was rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution was removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant was collected and a plaque assay was performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg, MD) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, MD, at pages 9-10.

Four days after serial dilution, the virus was added to the cells. After appropriate incubation, blue stained plaques are picked with the tip of an Eppendorf pipette. The agar containing the recombinant viruses was then resuspended in an Eppendorf tube containing 200 µl of Grace's medium. The agar is removed by a brief centrifugation and the supernatant containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they were stored at 4°C. A clone containing properly inserted soluble IL-1R AcM cDNA was identified by DNA analysis including restriction mapping and sequencing. This clone is designated herein as pA2HG16302.

Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus A2HG16302 at a multiplicity of infection ("MOI") of about 2 (about 1 to about 3). Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc.,

Gaithersburg, MD). 42 hours later, 5  $\mu$ Ci of  $^{35}$ S methionine and 5 MCi  $^{35}$ S cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation, lysed and the labeled proteins are visualized by SDS-PAGE and autoradiography.

5      ***Example 4: Tissue distribution of Soluble IL-1R AcM protein expression***

10      Northern blot analysis is carried out to examine the levels of expression of the gene encoding the IL-1R AcM protein in human tissues, using methods described by, among others, Sambrook *et al.*, cited above. A cDNA probe containing the entire nucleotide sequence of the IL-1R AcM protein of the present invention (SEQ ID NO:1) is labeled with  $^{32}$ P using the *rediprime*<sup>TM</sup> DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labelling, the probe was purified using a CHROMA SPIN-100<sup>TM</sup> column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labelled probe is then used to examine various human  
15      tissues for the expression of the gene encoding the IL-1R AcM protein.

20      Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) are obtained from Clontech and were examined with labelled probe using ExpressHyb<sup>TM</sup> Hybridization Solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70°C overnight, and films developed according to standard procedures.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

25      Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited in this application is hereby incorporated herein by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Bednarik, Daniel P.  
Olsen, Henrik S.  
Rosen, Craig A.
- (ii) TITLE OF INVENTION: Soluble Interleukin-1 Receptor Accessory  
Molecule
- (iii) NUMBER OF SEQUENCES: 30
- (iv) CORRESPONDENCE ADDRESS:
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  - (D) STATE: DC
  - (E) COUNTRY: USA
  - (F) ZIP: 20005-3934
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: To be assigned
  - (B) FILING DATE: Herewith
  - (C) CLASSIFICATION:
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  - (A) APPLICATION NUMBER: US 60/024,581
  - (B) FILING DATE: 26-AUG-1996
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Steffe, Eric K.
  - (B) REGISTRATION NUMBER: 36,688
  - (C) REFERENCE/DOCKET NUMBER: 1488.0450001/EKS/KMT
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 202-371-2600
  - (B) TELEFAX: 202-371-2540

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2155 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

08917710 082697

(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 303..1370

(ix) FEATURE:

(A) NAME/KEY: sig\_peptide  
(B) LOCATION: 303..353

(ix) FEATURE:

(A) NAME/KEY: mat\_peptide  
(B) LOCATION: 354..1370

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGGTGGCGCC CGTTCTAGAA CTAGTGGATC CCCCgggatg CAGGAATTCG GCACGAGAAA	60
GTGCGGCGGA AAGTAAGAGG CTCACTGGGG AAGACTGCCG GGATCCAGGT CTCCGGGGTC	120
CGCTTTGGCC AGAGGCGCGG AAGGAAGCAG TGCCCGGCGA CACTGCACCC ATCCCGGCTG	180
CTTTTGCTGC GCCCTCTCAG CTTCCCAAGA AAGGCATCGT CATGTGATCA TCACCTAAGA	240
ACTAGAACAT CAGCAGGCC TTAGAAGCCT CACTCTTGCC CCTCCCTTTA ATATCTCAAA	300
GG ATG ACA CTT CTG TGG TGT GTA GTG AGT CTC TAC TTT TAT GGA ATC	347
Met Thr Leu Leu Trp Cys Val Val Ser Leu Tyr Phe Tyr Gly Ile	
-17 -15 -10 -5	
CTG CAA AGT GAT GCC TCA GAA CGC TGC GAT GAC TGG GGA CTA GAC ACC	395
Leu Gln Ser Asp Ala Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr	
1 5 10	
ATG AGG CAA ATC CAA GTG TTT GAA GAT GAG CCA GCT CGC ATC AAG TGC	443
Met Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys	
15 20 25 30	
CCA CTC TTT GAA CAC TTC TTG AAA TTC AAC TAC AGC ACA GCC CAT TCA	491
Pro Leu Phe Glu His Phe Leu Lys Phe Asn Tyr Ser Thr Ala His Ser	
35 40 45	
GCT GGC CTT ACT CTG ATC TGG TAT TGG ACT AAG CAG GAC CGG GAC CTT	539
Ala Gly Leu Thr Leu Ile Trp Tyr Trp Thr Lys Gln Asp Arg Asp Leu	
50 55 60	
GAG GAG CCA ATT AAC TTC CGC CTC CCC GAG AAC CGC ATT AGT AAG GAG	587
Glu Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu	
65 70 75	
AAA GAT GTG CTG TGG TTC CGG CCC ACT CTC CTC AAT GAC ACT GGC AAC	635
Lys Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn	
80 85 90	
TAT ACC TGC ATG TTA AGG AAC ACT ACA TAT TGC AGC AAA GTT GCA TTT	683

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Tyr Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe	
95 100 105 110	
CCC TTG GAA GTT GTT CAA AAA GAC AGC TGT TTC AAT TCC CCC ATG AAA	731
Pro Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Pro Met Lys	
115 120 125	
CTC CCA GTG CAT AAA CTG TAT ATA GAA TAT GGC ATT CAG AGG ATC ACT	779
Leu Pro Val His Lys Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile Thr	
130 135 140	
TGT CCA AAT GTA GAT GGA TAT TTT CCT TCC AGT GTC AAA CCG ACT ATC	827
Cys Pro Asn Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Thr Ile	
145 150 155	
ACT TGG TAT ATG GGC TGT TAT AAA ATA CAG AAT TTT AAT AAT GTA ATA	875
Thr Trp Tyr Met Gly Cys Tyr Lys Ile Gln Asn Phe Asn Asn Val Ile	
160 165 170	
CCC GAA GGT ATG AAC TTG AGT TTC CTC ATT GCC TTA ATT TCA AAT AAT	923
Pro Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn	
175 180 185 190	
GGA AAT TAC ACA TGT GTT GTT ACA TAT CCA GAA AAT GGA CGT ACG TTT	971
Gly Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe	
195 200 205	
CAT CTC ACC AGG ACT CTG ACT GTA AAG GTA GTA GGC TCT CCA AAA AAT	1019
His Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn	
210 215 220	
GCA GTG CCC CCT GTG ATC CAT TCA CCT AAT GAT CAT GTG GTC TAT GAG	1067
Ala Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr Glu	
225 230 235	
AAA GAA CCA GGA GAG GAG CTA CTC ATT CCC TGT ACG GTC TAT TTT AGT	1115
Lys Glu Pro Gly Glu Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser	
240 245 250	
TTT CTG ATG GAT TCT CGC AAT GAG GTT TGG TGG ACC ATT GAT GGA AAA	1163
Phe Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys	
255 260 265 270	
AAA CCT GAT GAC ATC ACT ATT GAT GTC ACC ATT AAC GAA AGT ATA AGT	1211
Lys Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser	
275 280 285	
CAT AGT AGA ACA GAA GAT GAA ACT AGA ACT CAG ATT TTG AGC ATC AAG	1259
His Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys	
290 295 300	
AAA GTT ACC TCT GAG GAT CTC AAG CGC AGC TAT GTC TGT CAT GCT AGA	1307
Lys Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg	
305 310 315	

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AGT GCC AAA GGC GAA GTT GCC AAA GCA GCC AAG GTG AAG CAG AAA GGT	1355
Ser Ala Lys Gly Glu Val Ala Lys Ala Ala Lys Val Lys Gln Lys Gly	
320 325 330	
AAT AGA TGC GGT CAG TGATGAATCT CTCAGCTCCA AATTAACATT GTGGTGAATA	1410
Asn Arg Cys Gly Gln	
335	
AGGACAAAAG GAGAGATTGA GAACAAGAGA GCTCCAGCAC CTAGCCTGAC GGCATCTAAC	1470
CCATAGTAAT GAATCAAAC TAAATGAAAA ATATGAAAGT TTTTCATCTAT GTAAGATACT	1530
CAAAATATTG TTTCTGATAT TGTTAGTACC GTAATGCCCA AATGTAGCTA AAAAAATCGA	1590
CGTGAGTACA GTGAGACACA ATTTTGTGTC TGTACAATTA TGAAAAATTA AAAACAAAGA	1650
AAATATTCAA AGCTACCAAA GATAGAAAAA ACTGGTAGAG CCACATATTG TTGGTGAATT	1710
ATTAAGACCC TTTTAAAAAT CATTTCATGGT AGAGTTTAAAG AGTCATAAAA AAGATTGCAT	1770
CATCTGACCT AAGACTTTTCG GAATTTTTC TGAACAAATA ACAGAAAGGG AATTATATAC	1830
CTTTTAATAT TATTAGAAGC ATTATCTGTA GTTGTAAAAC ATTATTAATA GCAGCCATCC	1890
AATTGTATGC AACTAATTAA GGTATTGAAT GTTTATTTTC CAAAAATGCA TAATTATAAT	1950
ATTATTTTAA ACACTATGTA TCAATATTTA AGCAGGTTTA TAATATACCA GCAGCCACAA	2010
TTGCTAAAT GAAAATCATT TAAATTATGA TTTTAAATGG TATACACATG ATTTCTATGT	2070
TGATAGTACT ATATTATTCT ACAATAAATG GAAATTATAA AGCCTTCTTG TCAGAAGTGC	2130
TGCTCCTAAA AAAAAAAAAA AAAAA	2155

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 356 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Thr Leu Leu Trp Cys Val Val Ser Leu Tyr Phe Tyr Gly Ile Leu	
-17 -15 -10 -5	
Gln Ser Asp Ala Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met	
1 5 10 15	
Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro	
20 25 30	

Leu Phe Glu His Phe Leu Lys Phe Asn Tyr Ser Thr Ala His Ser Ala  
 35 40 45  
 Gly Leu Thr Leu Ile Trp Tyr Trp Thr Lys Gln Asp Arg Asp Leu Glu  
 50 55 60  
 Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys  
 65 70 75  
 Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr  
 80 85 90 95  
 Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro  
 100 105 110  
 Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Pro Met Lys Leu  
 115 120 125  
 Pro Val His Lys Leu Tyr Ile Glu Tyr Gly Ile Gln Arg Ile Thr Cys  
 130 135 140  
 Pro Asn Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Thr Ile Thr  
 145 150 155  
 Trp Tyr Met Gly Cys Tyr Lys Ile Gln Asn Phe Asn Asn Val Ile Pro  
 160 165 170 175  
 Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn Gly  
 180 185 190  
 Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe His  
 195 200 205  
 Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn Ala  
 210 215 220  
 Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr Glu Lys  
 225 230 235  
 Glu Pro Gly Glu Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser Phe  
 240 245 250 255  
 Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys  
 260 265 270  
 Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser His  
 275 280 285  
 Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys  
 290 295 300  
 Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg Ser  
 305 310 315  
 Ala Lys Gly Glu Val Ala Lys Ala Ala Lys Val Lys Gln Lys Gly Asn

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320

325

330

335

Arg Cys Gly Gln

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 350 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Gly Leu Leu Trp Tyr Leu Met Ser Leu Ser Phe Tyr Gly Ile Leu  
1 5 10 15

Gln Ser His Ala Ser Glu Arg Cys Asp Asp Trp Gly Leu Asp Thr Met  
20 25 30

Arg Gln Ile Gln Val Phe Glu Asp Glu Pro Ala Arg Ile Lys Cys Pro  
35 40 45

Leu Phe Glu His Phe Leu Lys Tyr Asn Tyr Ser Thr Ala His Ser Ser  
50 55 60

Gly Leu Thr Leu Ile Trp Tyr Trp Thr Arg Gln Asp Arg Asp Leu Glu  
65 70 75 80

Glu Pro Ile Asn Phe Arg Leu Pro Glu Asn Arg Ile Ser Lys Glu Lys  
85 90 95

Asp Val Leu Trp Phe Arg Pro Thr Leu Leu Asn Asp Thr Gly Asn Tyr  
100 105 110

Thr Cys Met Leu Arg Asn Thr Thr Tyr Cys Ser Lys Val Ala Phe Pro  
115 120 125

Leu Glu Val Val Gln Lys Asp Ser Cys Phe Asn Ser Ala Met Arg Phe  
130 135 140

Pro Val His Lys Met Tyr Ile Glu His Gly Ile His Lys Ile Thr Cys  
145 150 155 160

Pro Asn Val Asp Gly Tyr Phe Pro Ser Ser Val Lys Pro Ser Val Thr  
165 170 175

Trp Tyr Lys Gly Cys Thr Glu Ile Val Asp Phe His Asn Val Leu Pro  
180 185 190

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Glu	Gly	Met	Asn	Leu	Ser	Phe	Phe	Ile	Pro	Leu	Val	Ser	Asn	Asn	Gly	
		195						200					205			
Asn	Tyr	Thr	Cys	Val	Val	Thr	Tyr	Pro	Glu	Asn	Gly	Arg	Leu	Phe	His	
	210					215					220					
Leu	Thr	Arg	Thr	Val	Thr	Val	Lys	Val	Val	Gly	Ser	Pro	Lys	Asp	Ala	
225				230						235					240	
Leu	Pro	Pro	Gln	Ile	Tyr	Ser	Pro	Asn	Asp	Arg	Val	Val	Tyr	Glu	Lys	
			245						250					255		
Glu	Pro	Gly	Glu	Glu	Leu	Val	Ile	Pro	Cys	Lys	Val	Tyr	Phe	Ser	Phe	
			260					265					270			
Ile	Met	Asp	Ser	His	Asn	Glu	Val	Trp	Trp	Thr	Ile	Asp	Gly	Lys	Lys	
	275						280					285				
Pro	Asp	Asp	Val	Thr	Val	Asp	Ile	Thr	Ile	Asn	Glu	Ser	Val	Ser	Tyr	
	290					295					300					
Ser	Ser	Thr	Glu	Asp	Glu	Thr	Arg	Thr	Gln	Ile	Leu	Ser	Ile	Lys	Lys	
305				310					315						320	
Val	Thr	Pro	Glu	Asp	Leu	Arg	Arg	Asn	Tyr	Val	Cys	His	Ala	Arg	Asn	
			325					330						335		
Thr	Lys	Gly	Glu	Ala	Glu	Gln	Ala	Ala	Lys	Val	Lys	Gln	Lys			
			340				345						350			

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 294 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCTATGAGAA AGAACAAGGA GAGGAGCTAC TCATTCCCTG TACGGTCTAT TTTAGTTTTTC	60
TGATGGATTTC TCGCAATGAG GTTTGGTGGA CCATTGATGG AAAAAAACCT GATGACATCA	120
CTATTGATGT CACCATTAAC GAAAGTATAA GTCATAGTAG AACAGAAGAT GAAACAAGAA	180
CTCAGATTTT GAGCATCAAG AAAGTTACCT CTGAGGATCT CAAGCGCANT ANTGTCTGTC	240
ATGCTAGAAG TGCCAAAGGC GAAGTTGCCA AAGCAGCCAA GGTGAAGCAG AAAG	294

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 252 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGGAACCTCC AAACATATAG AAGTAAAGAC ACAGGGCTGT TATAAAATAC AGAATTTTAA 60  
TAATGTAATA CCCGAAGGTA TGANCTTGAG TTTCTCATT GCCTTAATTT CAAATAATGG 120  
AAATTACACA TGTNTTGTTA CATATCCAGA AAATGGACGT ACGTTTCATC TCACCAGGAC 180  
TCTGCTCTNT AAAGGTAGTA GGCTCTCCAA AAANTGCAGT GCCCCCTGTG ATCCATTCAC 240  
CTAATGATCA TG 252

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 425 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAGACAGCGT CTTGCTCTGT CACCTGGGCT GGAGTGCAGT GGCGAGATCT CGGCTCACTG 60  
CAACCTCTGC CTCCAGGTT CAAGCAATTC TCCTGCCTCA CCCTCCTGAG TAGCTGGGAT 120  
TACAGGTGTA TGCCACCATG CCGGCTAATT TTTGTATTTT CTAGTAGAGA CTAGGTTTCA 180  
CCATGTTGGC CAGGCTGGTC TTGAACTATT TTTTTTCTT TTTCTCGTGC CGAATTCCTG 240  
CAGCCCGGGG GATCCACTAG TTCTAGAGCG GCCGCCACCG CGGTGGAGCT CCAGCTTTTG 300  
TTCCCTTTAG TGAGGGNTAA TTTCGAGCTT GGC GTAATCA TGGTCATAGC TGTTTCCTGT 360  
GTGAAATTGT TATCCGCTCA CAATTTTACA CAACATACGA GCCGGAAGCA TAAAGTGTA 420

AGCCT

425

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 80 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCACNNAAGG GACAAAAGCT GGAGCTCCAC CGCGGGCNGC NCGTTCTAGA ACTAGTGGAT 60  
CCCCCGGGCT GCAGGAATTC 80

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 100 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAACTAGTGG ATCCCCCGGG CTGCAGGAAT TCGNCACGAG ACCANCTCAC CTTTCCCCAC 60  
ACTAGCTCAN GNACAGACAG ANTGGACTAA AAATAGTTGA 100

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 262 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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GNCTAGAACT AGTGGATCCC CCGGGCTGCA GGAATTCGGC ACGAGGGGCT GCTCGAGCTG	60
CCAACAACGG AGCATTGCCC CCTGGACCTN AGCTGACATC GTGCGTAGNC TAGGCATGNG	120
TGGTTGTAGG GACTTACGTC TTTCTACCNT GANNACGGT TATCACTGNC GANGTCCACC	180
CACCGGGGNT GNNCAACTTN CGGNGGAAGG TACTACNTAC TTTCAAACCC CCTAACTTGT	240
TCCTTTTTTTG CAGGATCGAG TN	262

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 401 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

NTTAGTGTAC AGACACAAAA TTGTGTCTCA CTGTACTCAC GTCGATTTTT TTAGCTACAT	60
TTGGGCATTA CGGTACTAAC AATATCAGAA ACAATATTTT GAGTATCTTA CATAGATGAA	120
AACTTTCATA TTTTTCATTT AAGTTTGATT CATTACTATG GGTTAGATGC CGTCGGNTAG	180
GTGCTGGAGC TCTCTTGTTT TCAATCTCTC CTTTGTCTCT TATTCACCAC AATGTTAATT	240
TGGAGCTGAG AGATTCATCA CTGACCGCAT CTATTACCTT TCTGCTTCAC CTTGGCTGCT	300
TTGGCAACTT CGCCTTTGGA CCTTCTAGCA TGACAGACAT AGCTGCGCTT GAGATCCTCA	360
GAGGTAAGTT TCTTGANGCT CAAAATCTGA GTTCTTGTTTT C	401

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 443 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CATAATTGTA CAGACACAAA ATTGTGTCTC ACTGTACTCA CGTCGATTTT TTTAGCTACA	60
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TTTGGGCATT ACGGTACTAA CAATATCAGA AACAAATATTT TGAGTATCTT ACATAGATGA 120  
AAACTTTCAT ATTTTTCATT TAAGTTTNGA TTCATTACTA TGGGTTAGAT GCCGTCGGGC 180  
TNAGGTGCTG GAGCTCTCTT GTTCTCAATC TCTCCTTTTG TCCTTATTCA CCACAATGTT 240  
AATTTGGAGC TGAGAGATTC ATCACTGACC GCATCTATTA CCTTTCTGCT TCACCTTGGC 300  
TCGCTTTGGC AACTTCGCCT TTGGACTTCT AGCATGACAG ACATAGCTGC GCTTGGAGAT 360  
CCTCAGAGGT AACTTTCTTG ATGGCTCAAA ATCNGAGTTC TTGTTTCATC TTCTGTTCTA 420  
CTATGACTTA TACTTTCGTT AAN 443

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 319 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CATAATTGTA CAGACACAAA ATTGTGCTCA CTGTACNCAC GTCGATTTTT TTAGCTACAT 60  
TTGGGCATTA CGGTACTAAC AATATCAGAA ACAATATTTT GAGTATCTTA CATAGATGAA 120  
AACTTTCATA TTTTTCATTT AAGTTTGATT CATTACTATG GGTTAGATGC CGTCGGGCTA 180  
GGGGCTGGAG CTCTCTTGTT CTCAATCTCT CCTTTTGTCC TTATTCACCA CAATGTTAAT 240  
TTGGNGCTGA GAGATTCATC ACTGACCGCA TCTATTACCN TTCTGCTTCA NCTTGGCTGC 300  
TTTGGNAACT TCGNCTTTG 319

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 396 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

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TCTATGAGAA AGAACCAGGA GAGGAGCTAC TCATTCCCTG TACGGTCTAT TTTAGTTTTC	60
TGATGGATTG TCGCAATGAG GTTTGGTGGA CCATTGATGG AAAAAACCT GATGACATCA	120
CTATTGATGT CACCATTAAC GAAAGTATAA GTCATAGTAG AACAGAAGAT GAAACAAGAA	180
CTCAGATTTT GAGCATCAAG AAAGTTACCT CTGAGGATCT CAAGCGCANT ANTGTCTGTC	240
ATGCTAGAAG TGCCAAAGGC GAAGTTGCCA AAGCAGCCAA GGTGAAGCAG AAAGTGCCAG	300
CTCCAAGATA CACAGTGGA CTGGCTTGTG GTTTTGGAGC CACAGTCCTG CTAGTGGTGA	360
TTCTCATTGT TGTTTACCAT GTTTACTTGG CTAGAG	396

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 426 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCTCTCCAAA AAATGCAGTG CCCCTGTGA TCCATTCACC TAATGATCAT GTGGTCTATG	60
AGAAAGAACC AGGAGAGGAG CTACTCATTC CCTGTACGGT CTATTTTAGT TTTCTGATGG	120
ATTCTCGCAA TGAGGTTTGG TGGACCATTG ATGGAAAAAA ACCTGATGAC ATCACTATTG	180
ATGTCACCAT TAACGAAAGT ATAAGTCATA GTAGAACAGA AGATGAAACA AGAACTCAGA	240
TTTTGAGGCA TCAAGAAAGT TACCTCTGAG GATCTCAAGC GCNTAATNGT CTGTNCATGG	300
CTAGGAAGTG CCAAAGNGGA AGTTGGCCAA AGGCAGCCAA GGTNGAGGCA GGAAAGGTTA	360
TTAGGTGGCG GTTCAGTTGA TGGATTCTCT TCAGGNTCCC AATTTTAACN TTGTTGGGTG	420
GATTTA	426

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 570 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TTCGGCACAG GGAACCTCCA AACATATAGA AGTAAAGACA CAGGGCTGTT ATAAAATACA	60
GAATTTTAAT AATGTAATAC CCGAAGTATG AACTTGAGTT TCCTCATTGC CTTAATTTCA	120
AATAATGGAA ATTACACATG TGTGTTTACA TATCCAGAAA ATGGACGTAC GTTTCATCTC	180
ACCAGGACTC TGAAGTATAA GGTAGTAGGC TCTCCAAAAA ATGCAGTGCC CCCTGTGATC	240
CATTACCTA ATGATCATGT GGTCTATGAG AAAGAACCAG GGAGAGTAGC TACTCATTCC	300
CTGTACGGTC TATTTTAGTT TTCTGATGGA TTCTCGCAAT GGAGGTTTNG TGGGACCATT	360
TGATGGGAAA AAAACCTGGA TNGACATCAN TATTTGATGT TCACCATTTA ACGGAAAGTA	420
TTAAGTCCTT AGTTAGGANC AGGTGGTTGA ANACAGGAAN TCCGGTTTTT GAGGCTTCAG	480
GAAAGTTTAC CCCTGNGGGT TCTTCAGGNG CCGATTGTTN TGTTTNTTGT TNGGAGGTGN	540
CCCAGNGGAA GTTTTGNCCA AGGCGGCCAG	570

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 256 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TAGTACTATC AACATAGAAA NCATGTTTAT ACCATTTAAA ATCATAATTT AAATGATTTT	60
CATTTTAGCA ATTGTGGCTG CTGGTATATT ATAAACCTGC TTAAATATTG ATACATAGTG	120
TTTAANATAA TATTATAATT CTGCATTTTT GGAAATAAAA CATTCAATAC CTTAATTAGT	180
TGCATACAAT TGGATGGCTG CTATTAATAA NGTTTTACAA CTACAGATAA TGCTTCTAAT	240
ANTATTANCG GGNATA	256

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 333 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GAACAGAAGA TGAAACAAGA ACTCAGATTT TGAGCATCAA GAAAGTTACC TCTGAGGATC	60
TCAAGCGCAN TATTGTCTGT CATGCTAGAA GTGCCAAAGG GAANGTTGCC AAAGCAGCCA	120
AGGTGAAGCA GAAAGGTAAT AGATGCGGTC AGTGATGAAT CTCTCAGCTC CAAATTAACA	180
TTGTGGGTGA ATAAGGACAA AAGGAGAGAT TGAGGAACAA GAGAGCTCCA GCACCTAGCC	240
TGACGGCATC TTAACCCCAT AGTAATTGAA TCCAAC TTTA AATGGAAAAN TTTGNAGTTT	300
TTTCATCCTT NGGTAGGGTA CTTCAANTTT TGT	333

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 271 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TTCTGACAAG AAGGCTTTAT AATTTCCATT TATTGTAGAA TAATATAGTA CTATCAACAT	60
AGAAATCATG TGTATACCAT TTAAAATCAT AATTTAAATG ATTTTCATTT TAGCAATTGT	120
GGCTGCTGGG ATATTATAAA CCTGCTTAAA TATTGATACA TAGNGTTTAA AATAATATTA	180
TAATTATGCA NTTTTGGGGA AATAAACATT CAATACCCNT AATAGGTGCA TACAATTGGG	240
AGGGCTGCNA TTAATAATGG TTTCCACNAC C	271

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 214 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

0891710-08969

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TATTTTCCAA AAATGCATAA TTATAATATT ATTTYAMMCA CTATGTATCA ATATTTAAGC	60
AGGTTTATAA TATACCAGCA GCCACAATTG CTAAAATGAA AATCATTAA ATTATGATTT	120
TAAATGGTAT ACACATGATT YCTATGTTGA TAGTACTATA TTATTCTACA ATAAATGGAA	180
ATTATAACGC CTTCTTGTC GAAGTGCTGC TCCT	214

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 309 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCTTTATAAT ATTTTANTTA TTGTAGAATA ATATAGTACT ATCAACATAG AAATCATGTG	60
TATACCATTT AAAATCATAA TTAAATGAT TTTCATTTTA GCAATTGTGG CTGCTGGGTA	120
TATTATAAAC CTGCTTAAAT ATTGATACAT AGTGTTTAAA ATAATATTAT AATTATGGCA	180
TTTTTGGGAA ATAAACATTC AATACCTTAA TTGGNTGGCA TACAATGGGG TGGGCNGGCT	240
ATTAATAATG GTTTTACAAC TACAGGGTAA TGGCNTCCTA ATAATATTAA AGGGGGGNIA	300
TAATCCCC	309

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 486 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

0091710-0899

(2) INFORMATION FOR SEQ ID NO:22:

(A) LENGTH: 159 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

(2) INFORMATION FOR SEQ ID NO:23:

SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

25

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GTCGACTCAC TGACCGCATC T

21

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 35 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGATCCATCC GCCATCATGA CACTTCTGTG GTGTG

35

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 47 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TCTAGAAAAG CGTAGTCTGG GACGTCGTAT GGGTACTGAC CGCATCT

47

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 35 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

0891710-082697

GACTGGATCC GCCATCATGA CACTTCTGTG GTGTG

35

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GAATTCCTCA CTGACCGCAT CT

22

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 35 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GACTGGATCC GCCATCATGA CACTTCTGTG GTGTG

35

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 31 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GACTGGTACC CATAGAAATC ATGTGTATAC C

31

00012710 08259

***What Is Claimed Is:***

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

5 (a) a nucleotide sequence encoding a polypeptide comprising amino acids from about -17 to about 339 in SEQ ID NO:2;

(b) a nucleotide sequence encoding a polypeptide comprising amino acids from about -16 to about 339 in SEQ ID NO:2;

10 (c) a nucleotide sequence encoding a polypeptide comprising amino acids from about 1 to about 339 in SEQ ID NO:2;

(d) a nucleotide sequence encoding a polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97666;

15 (e) a nucleotide sequence encoding the mature IL-1R AcM polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97666; and

(f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d) or (e).

20 2. An isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to a polynucleotide having a nucleotide sequence identical to a nucleotide sequence in (a), (b), (c), (d) or (e) of claim 1 wherein said polynucleotide which hybridizes does not hybridize under stringent hybridization conditions to a polynucleotide having a nucleotide sequence consisting of only A residues or of only T residues.

25 3. An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of a IL-1R

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AcM polypeptide having an amino acid sequence in (a), (b), (c), (d), or (e) of claim 1.

4. The isolated nucleic acid molecule of claim 3, which encodes an epitope-bearing portion of a IL-1R AcM polypeptide selected from the group consisting of: a polypeptide comprising amino acid residues from about 6 to about 15 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 57 to about 66 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 70 to about 79 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 106 to about 112 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 115 to about 124 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from about 129 to about 135 in SEQ ID NO:2.

5. An isolated nucleic acid molecule, comprising a polynucleotide having a sequence selected from the group consisting of the nucleotide sequence of a fragment of the sequence shown in SEQ ID NO:1 or the complement thereof, wherein said fragment comprises at least 50 contiguous nucleotides of SEQ ID NO:1, provided that said fragment does not have a sequence starting:

at nucleotide 1 and ending at nucleotide 60 of SEQ ID NO:1 or any subfragment thereof or the complement thereof;

at nucleotide 139 and ending at nucleotide 214 of SEQ ID NO:1 or any subfragment thereof or the complement thereof;

at nucleotide 298 and ending at nucleotide 366 of SEQ ID NO:1 or any subfragment thereof or the complement thereof;

at nucleotide 839 and ending at nucleotide 1632 of SEQ ID NO:1 or any subfragment thereof or the complement thereof; or

at nucleotide 1922 and ending at nucleotide 2137 of SEQ ID NO:1, or any subfragment thereof or the complement thereof.

6. A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim 1 into a vector.

7. A recombinant vector produced by the method of claim 6.

8. A method of making a recombinant host cell comprising introducing the recombinant vector of claim 7 into a host cell.

9. A recombinant host cell produced by the method of claim 8.

10. A recombinant method for producing a IL-1R AcM polypeptide, comprising culturing the recombinant host cell of claim 9 under conditions such that said polypeptide is expressed and recovering said polypeptide.

11. An isolated IL-1R AcM polypeptide having an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

- (a) amino acids from about -17 to about 339 in SEQ ID NO:2;
- (b) amino acids from about -16 to about 339 in SEQ ID NO:2;
- (c) amino acids from about 1 to about 339 in SEQ ID NO:2;
- (d) the amino acid sequence of the IL-1R AcM polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97666;
- (e) the amino acid sequence of the mature IL-1R AcM polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97666; and
- (f) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d) or (e).

12. An isolated polypeptide comprising an epitope-bearing portion of the IL-1R AcM protein, wherein said portion is selected from the group

consisting of: a polypeptide comprising amino acid residues from about 6 to about 15 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 57 to about 66 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 70 to about 79 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 106 to about 112 in SEQ ID NO:2; a polypeptide comprising amino acid residues from about 115 to about 124 in SEQ ID NO:2; and a polypeptide comprising amino acid residues from about 129 to about 135 in SEQ ID NO:2.

13. The isolated polypeptide of claim 11, which is produced or contained in a recombinant host cell.

14. The isolated polypeptide of claim 11, wherein said recombinant host cell is mammalian.

15. An isolated antibody that binds specifically to an IL-1R AcM polypeptide of claim 11.

16. An isolated nucleic acid molecule comprising a polynucleotide encoding an IL-1R AcM polypeptide wherein, except for at least one to fifty conservative amino acid substitutions, said polypeptide has a sequence selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide comprising amino acids from about -17 to about 339 in SEQ ID NO:2;

(b) a nucleotide sequence encoding a polypeptide comprising amino acids from about -16 to about 339 in SEQ ID NO:2;

(c) a nucleotide sequence encoding a polypeptide comprising amino acids from about 1 to about 339 in SEQ ID NO:2;

(d) a nucleotide sequence encoding a polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97666;

(e) a nucleotide sequence encoding the mature IL-1R AcM polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97666; and

(f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d) or (e).

17. An isolated IL-1R AcM polypeptide wherein, except for at least one to fifty conservative amino acid substitutions, said polypeptide has a sequence selected from the group consisting of:

(a) amino acids from about -17 to about 339 in SEQ ID NO:2;  
(b) amino acids from about -16 to about 339 in SEQ ID NO:2;  
(c) amino acids from about 1 to about 339 in SEQ ID NO:2;  
(d) the amino acid sequence of the IL-1R AcM polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97666;

(e) the amino acid sequence of the mature IL-1R AcM polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97666; and

(f) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d) or (e).

18. A method for identifying a type I IL-1 receptor agonist, comprising:

(a) providing a host cell containing recombinant genes which express a polypeptide comprising a type I IL-1 receptor and a polypeptide comprising the polypeptide of claim 16, wherein said type I IL-1R and the polypeptide of claim 16 form a complex;

(b) administering a candidate agonist to said cell; and

(c) determining the binding affinity of said complex for said candidate agonist relative to the binding affinity of said complex for IL-1.

19. A method for identifying a type I IL-1 receptor antagonist comprising:

(a) providing a host cell containing recombinant genes which express a polypeptide comprising a type I IL-1 receptor and a polypeptide comprising the polypeptide of claim 16, wherein said type I IL-1R and the polypeptide of claim 16 form a complex;

(b) administering a candidate antagonist to said cell; and

(c) determining the whether said candidate agonist disrupts or prevents formation of a complex between type I IL-1R and the polypeptide of claim 16.

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***Soluble Interleukin-1 Receptor Accessory Molecule***

***Abstract***

The present invention relates to a novel soluble IL-1 receptor accessory molecule (IL-1R AcM) protein which is a member of the Ig superfamily. In particular, isolated nucleic acid molecules are provided encoding the human IL-1R AcM protein. IL-1R AcM polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. Screening methods are further provided for identifying agonist and antagonists of IL-1 signal transduction. The invention further relates to methods for treating physiologic and pathologic disease conditions with IL-1R AcM antagonists.

45-01.APL

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Figure 1

1 CGGTGGCGCCCGTTCTAGAACTAGTGGATCCCCCGGGATGCAGGAATTCGGGCACGAGAAA 60  
61 GTGCGGCGGAAAGTAAGAGGCTCACTGGGGAAGACTGCCGGGATCCAGGTCTCCGGGGTC 120  
121 CGCTTTGGCCAGAGGCGCGGAAGGAAGCAGTGCCCGGCGACACTGCACCCATCCCGGCTG 180  
181 CTTTTGCTGCGCCCTCTCAGCTTCCCAAGAAAGGCATCGTCATGTGATCATCACCTAAGA 240  
241 ACTAGAACATCAGCAGGCCCTTAGAAGCCTCACTCTTGCCCCCTCCCTTTAATATCTCAA 300  
301 GGATGACACTTCTGTGGTGTGTAGTGAGTCTCTACTTTTATGGAATCCTGCAAAGTGATG 360  
M T L L W C V V S L Y F Y G I L O S D A  
361 CCTCAGAACGCTGCGATGACTGGGGACTAGACACCATGAGGCAAATCCAAGTGTGTTGAAG 420  
S E R C D D W G L D T M R Q I Q V F E D  
421 ATGAGCCAGCTCGCATCAAGTGCCCACTCTTTGAACACTTCTTGAAATTCAACTACAGCA 480  
E P A R I K C P L F E H F L K F N Y S T  
481 CAGCCCATTCAGCTGGCCTTACTCTGATCTGGTATTGGACTAAGCAGGACCGGGACCTTG 540  
A H S A G L T L I W Y W T K Q D R D L E  
541 AGGAGCCAATTAACCTCCGCTCCCGGAGAACCGCATTAGTAAGGAGAAAGATGTGCTGT 600  
E P I N F R L P E N R I S K E K D V L W  
601 GGTTCGCGCCCACTCTCCTCAATGACACTGGCAACTATACCTGCATGTTAAGGAACACTA 660  
F R P T L L N D T G N Y T C M L R N T T  
661 CATATTGCAGCAAAGTTGCATTTCCCTTGGAAGTTGTTCAAAAAGACAGCTGTTTCAATT 720  
Y C S K V A F P L E V V Q K D S C F N S  
721 CCCCCATGAAACTCCAGTGACATAAACTGTATAGAATATGGCATTTCAGAGGATCACTT 780  
P M K L P V H K L Y I E Y G I Q R I T C  
781 GTCCAAATGTAGATGGATATTTTCTTCCAGTGTCAAACCGACTATCACTTGGTATATGG 840  
P N V D G Y F P S S V K P T I T W Y M G  
841 GCTGTTATAAAATACAGAATTTTAATAATGTAATACCCGAAGGTATGAACTTGAGTTTCC 900  
C Y K I Q N F N N V I P E G M N L S F L  
901 TCATTGCCTTAATTTCAAATAATGGAAATTACACATGTGTTGTTACATATCCAGAAAATG 960  
I A L I S N N G N Y T C V V T Y P E N G  
961 GACGTACGTTTCATCTCACCAGGACTCTGACTGTAAAGGTAGTAGGCTCTCCAAAAAATG 1020  
R T F H L T R T L T V K V V G S P K N A  
1021 CAGTGCCCCCTGTGATCCATTACCTAATGATCATGTGGTCTATGAGAAAGAACCAGGAG 1080  
V P P V I H S P N D H V V Y E K E P G E  
1081 AGGAGCTACTCATTCCTGTACGGTCTATTTTAGTTTTCTGATGGATTCTCGCAATGAGG 1140  
E L L I P C T A C Y F S F L M D S R N E V  
1141 TTTGGTGGACCATTGATGGAAAAAAACCTGATGACATCACTATTGATGTCACCATTAACG 1200  
W W T I D G K K P D D I T I D V T I N E  
1201 AAAGTATAAGTCATAGTAGAACAGAAGATGAAACTAGAACTCAGATTTTGAGCATCAAGA 1260  
S I S H S R T E D E T R T Q I L S I K K  
1261 AAGTTACCTCTGAGGATCTCAAGCGCAGCTATGTCTGTCATGCTAGAAGTGCCAAAGGCG 1320  
V T S E D L K R S Y V C H A R S A K G E  
1321 AAGTTGCCAAAGCAGCCAAGGTGAAGCAGAAAGGTAATAGATGCGGTCACTGATGAATCT 1380  
V A K A A K V K Q K G N R C G Q \*  
1381 CTCAGCTCCAAATTAACATTGTGGTGAATAAGGACAAAAGGAGAGATTGAGAACAAGAGA 1440  
1441 GCTCCAGCACCTAGCCTGACGGCATCTAACCATAGTAATGAATCAAACCTAAATGAAAA 1500  
1501 ATATGAAAGTTTTTCATCTATGTAAGATACTCAAATATTTGTTTCTGATATTGTTAGTACC 1560  
1561 GTAATGCCCAAATGTAGCTAAAAAAATCGACGTGAGTACAGTGAGACACAATTTTGTGTC 1620  
1621 TGTACAATTATGAAAAATTAAAAACAAAGAAATATTCAAAGCTACCAAAGATAGAAAAA 1680  
1681 ACTGGTAGAGCCACATATTGTTGGTGAATTATTAAGACCTTTTAAAAATCATTATGTTG 1740  
1741 AGAGTTTAAGAGTCATAAAAAAGATTGCATCATCTGACCTAAGACTTTTCGGAATTTTCC 1800  
1801 TGAACAAATAACAGAAAGGAATTATATACCTTTTAAATATTATTAGAAGCATTATCTGTA 1860  
1861 GTTGTAACAAATTATTAATAGCAGCCATCCAATTGTATGCAACTAATTAAGGTATTGAAT 1920  
1921 GTTTATTTTCCAAAAATGCATAATTATAATATTATTTTAAACACTATGTATCAATATTTA 1980  
1981 AGCAGGTTTATAATATACAGCAGCCACAATTGCTAAAATGAAAATCAATTAAATTATGA 2040  
2041 TTTTAAATGGTATACATGATTTCTATGTGTGATAGTACTATATTATTCTACAATAAATG 2100  
2101 GAAATTATAAAGCCTTCTTGTGTCAGAAGTGCTGCTCCTAAAAA 2155

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Figure 2A

Query: 303 MTLWCVVSLEYFYGILQSDASERCDDWGLDITMRQIQVFEDEPARIKCPLFEHFLKFNYST 482  
M LLW ++SL FYGILQS ASERCDDWGLDITMRQIQVFEDEPARIKCPLFEHFLK+NYST  
Sbjct: 1 MGLLWYIMSLSFYGILQSHASERCDDWGLDITMRQIQVFEDEPARIKCPLFEHFLKYNYST 60

Query: 483 AHSAGLTLIWIWIKQDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNIT 662  
AHS+GLTLIWIWT+QDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNIT  
Sbjct: 61 AHSSGLTLIWIWTRQDRDLEEPINFRLPENRISKEKDVLWFRPTLLNDTGNYTCMLRNIT 120

Query: 663 YCSKVAFPLEVVQKDSFCNSPMKLPVHKLYIEYGIQRITCPNVDGYFPSSVKPTITWYMG 842  
YCSKVAFPLEVVQKDSFCNS M+ PVHK+YIE+GI +ITCPNVDGYFPSSVKP++TWY G  
Sbjct: 121 YCSKVAFPLEVVQKDSFCNSAMRFPVHKMYIEHGIHKITCPNVDGYFPSSVKPSVTWYKG 180

Query: 843 CYKIQNFNNVIPEGMNLISFLIALISNNGNYTCVVITYPENGRTFHLTRTLTVKVVGSPKNA 1022  
C +I +F+NV+PEGMNLIS I L+SNNGNYTCVVITYPENGR FHLTRT+TVKVVGSPK+A  
Sbjct: 181 CTEIVDFHNVLPFGMNLISFFIPLVSNNNGNYTCVVITYPENGRFHLTRTVTVKVVGSPKDA 240

Query: 1023 VPPVIHSPNDHVVEKEPGEELLIPCTVYFSFLMDSRNEVWWTIDGKKPDDITIDVTINE 1202  
+PP I+SPND VVYEKEPGEEL+IPC VYFSF+MDS NEVWWTIDGKKPDD+T+D+TINE  
Sbjct: 241 LPPQIYSPNDRVVYEKEPGEELVIPCKVYFSFLMDSHNEVWWTIDGKKPDDVTVDITINE 300

Query: 1203 SISHSRTEDETRTQILSIKKVTSEDLKRSYVCHARSAKGEVAKAAKVQK 1352  
S+S+S TEDETRTQILSIKKVT EDL+R+YVCHAR+ KGE +AAKVQK  
Sbjct: 301 SVSYSSTEDETRTQILSIKKVTPEDLRNYVCHARNTKGEAEQAQAAKVQK 350

0891710-08269



Figure 2B

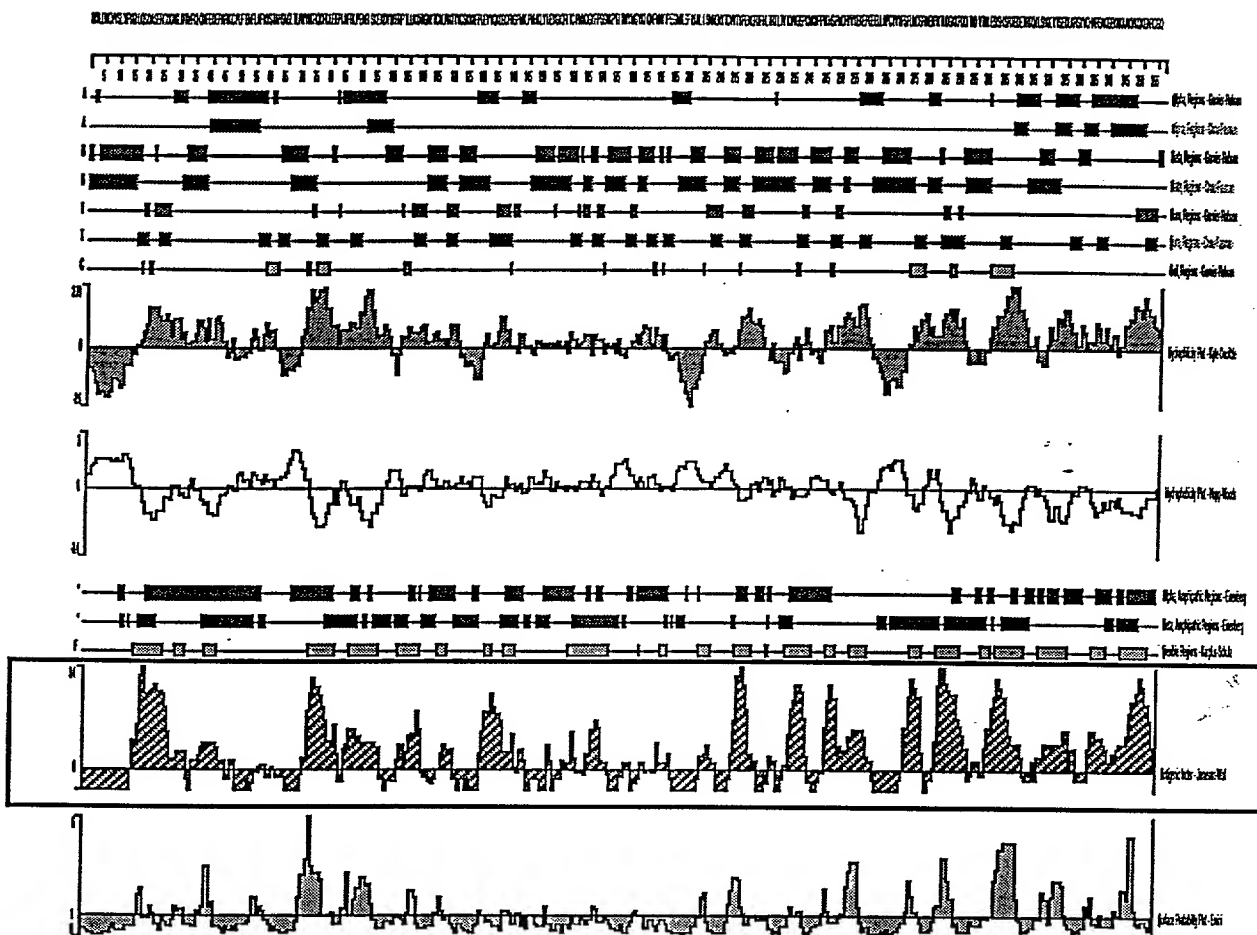
```

1  TCTATGAGAAAGAACCAGGAGAGGAGCTACTCATTCCCTGTACGGTCTAT 50
   |||
1060 TCTATGAGAAAGAACCAGGAGAGGAGCTACTCATTCCCTGTACGGTCTAT 1109
      51  TTTAGTTTTCTGATGGATTCTCGCAATGAGGTTTGGTGGACCATTGATGG 100
         |||
1110  TTTAGTTTTCTGATGGATTCTCGCAATGAGGTTTGGTGGACCATTGATGG 1159
      101  AAAAAAACCTGATGACATCACTATTGATGTCACCATTAACGAAAGTATAA 150
         |||
1160  AAAAAAACCTGATGACATCACTATTGATGTCACCATTAACGAAAGTATAA 1209
      151  GTCATAGTAGAACAGAAGATGAAACAAGAACTCAGATTTTGAGCATCAAG 200
         |||
1210  GTCATAGTAGAACAGAAGATGAAACAAGAACTCAGATTTTGAGCATCAAG 1259
      201  AAAGTTACCTCTGAGGATCTCAAGCGCANTANTGTCTGTCATGCTAGAAG 250
         |||
1260  AAAGTTACCTCTGAGGATCTCAAGCGCAGCTATGTCTGTCATGCTAGAAG 1309
      251  TGCCAAAGGCGAAGTTGCCAAAGCAGCCAAGGTGAAGCAGAAAG 294
         |||
1310  TGCCAAAGGCGAAGTTGCCAAAGCAGCCAAGGTGAAGCAGAAAG 1353

```

08917710.082697

Figure 3



MTLLWCVVSLYFYGILQSDASERCDDWGLDTMRQIQVFEDEPARIKCPLFEHFL  
 KFNYSTAHSAGLTLIWYWTRQDRDLEEPINERLPENRISKEKDVLWFRPTLLND  
 TGN<sup>Y</sup>TCMLRNTTYCSKVAFPLEVVQKDSCFNSPMKLPVHKLYIEYGIQRITCPN  
 VDGYFPSSVKPTITWYMGCYKIQNFNNVIPEGMNLSFLIALISNNGNYTCVVTP  
 ENGRTEHLTRTLTVKVVGSPKNAVPPVIHSPNDHVVEKEPGEELLIPCTVYFSF  
 LMDSRNEVWWTIDGKKPDDITIDVTINESISHSRTEDETRTQILSIKKVTSEDLKR  
 SYVCHARSAKGEVAKAAKVQKGNRCGQ.